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<p>(21) International Application Number: PCT/US98/06421</p> <p>(22) International Filing Date: 31 March 1998 (31.03.98)</p> <p>(30) Priority Data:</p> <table border="0"> <tr> <td>08/831,310</td> <td>1 April 1997 (01.04.97)</td> <td>US</td> </tr> <tr> <td>08/834,666</td> <td>1 April 1997 (01.04.97)</td> <td>US</td> </tr> </table> <p>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications</p> <table border="0"> <tr> <td>US</td> <td>08/831,310 (CON)</td> </tr> <tr> <td>Filed on</td> <td>1 April 1997 (01.04.97)</td> </tr> <tr> <td>US</td> <td>08/834,666 (CON)</td> </tr> <tr> <td>Filed on</td> <td>1 April 1997 (01.04.97)</td> </tr> </table> <p>(71) Applicants (<i>for all designated States except US</i>): MERIEUX ORAVAX SOCIETE EN NOM COLLECTIF, PASTEUR MERIEUX SERUMS ET VACCINS S.A. [FR/FR]; 58, avenue Leclerc, F-69007 Lyon (FR). HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).</p>		08/831,310	1 April 1997 (01.04.97)	US	08/834,666	1 April 1997 (01.04.97)	US	US	08/831,310 (CON)	Filed on	1 April 1997 (01.04.97)	US	08/834,666 (CON)	Filed on	1 April 1997 (01.04.97)	<p>(72) Inventors; and (75) Inventors/Applicants (<i>for US only</i>): KLEANTHOS, Harold [GB/US]; 89 Madison Avenue, Newtonville, MA 02160 (US). LISSOLO, Ling [FR/FR]; 691, rue du Vallor, F-69280 Marcy l'Etoile (FR). TOMB, Jean-François [-/US]; 3501 St. Paul Street, Baltimore, MD 21222 (US). MILLER, Charles [US/US]; 32 Maple Avenue, Medford, MA 02155 (US). AL-GARAWI, Amal [SA/US]; 32 Garrison Street, No. 4501, Boston, MA 02114 (US).</p> <p>(74) Agent: CLARK, Paul, T.; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110-2214 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
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<p>(54) Title: 76 kDa, 32 kDa, AND 50 kDa <i>HELICOBACTER</i> POLYPEPTIDES AND CORRESPONDING POLYNUCLEOTIDE MOLECULES</p> <p>(57) Abstract</p> <p>The invention provides 76 kDa, 32 kDa, and 50 kDa <i>Helicobacter</i> polypeptides, which can be used in vaccination methods for preventing or treating <i>Helicobacter</i> infection, and polynucleotides that encode these polypeptides. The invention also provides diagnostic methods employing these polypeptides.</p>																

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76 kDa, 32 kDa, and 50 kDa *HELICOBACTER* POLYPEPTIDES AND
CORRESPONDING POLYNUCLEOTIDE MOLECULES

- 5 The invention relates to *Helicobacter* polypeptides and corresponding polynucleotide molecules that can be used in methods to prevent or treat *Helicobacter* infection in mammals, such as humans.

Background of the Invention

- Helicobacter* is a genus of spiral, gram-negative bacteria that
10 colonize the gastrointestinal tracts of mammals. Several species colonize the stomach, most notably *H. pylori*, *H. heilmanii*, *H. felis*, and *H. mustelae*. Although *H. pylori* is the species most commonly associated with human infection, *H. heilmanii* and *H. felis* have also been isolated from humans, but at lower frequencies than *H. pylori*. *Helicobacter* infects over 50% of adult
15 populations in developed countries and nearly 100% in developing countries and some Pacific rim countries, making it one of the most prevalent infections worldwide.

- Helicobacter* is routinely recovered from gastric biopsies of humans with histological evidence of gastritis and peptic ulceration. Indeed, *H. pylori*
20 is now recognized as an important pathogen of humans, in that the chronic gastritis it causes is a risk factor for the development of peptic ulcer diseases and gastric carcinoma. It is thus highly desirable to develop safe and effective vaccines for preventing and treating *Helicobacter* infection.

- A number of *Helicobacter* antigens have been characterized or
25 isolated. These include urease, which is composed of two structural subunits of approximately 30 and 67 kDa (Hu *et al.*, Infect. Immun. 58:992, 1990; Dunn *et al.*, J. Biol. Chem. 265:9464, 1990; Evans *et al.*, Microbial Pathogenesis 10:15,

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1991; Labigne *et al.*, J. Bact., 173:1920, 1991); the 87 kDa vacuolar cytotoxin (VacA) (Cover *et al.*, J. Biol. Chem. 267:10570, 1992; Phadnis *et al.*, Infect. Immun. 62:1557, 1994; WO 93/18150); a 128 kDa immunodominant antigen associated with the cytotoxin (CagA, also called TagA; WO 93/18150; U.S. Patent No. 5,403,924); 13 and 58 kDa heat shock proteins HspA and HspB (Suerbaum *et al.*, Mol. Microbiol. 14:959, 1994; WO 93/18150); a 54 kDa catalase (Hazell *et al.*, J. Gen. Microbiol. 137:57, 1991); a 15 kDa histidine-rich protein (Hpn) (Gilbert *et al.*, Infect. Immun. 63:2682, 1995); a 20 kDa membrane-associated lipoprotein (Kostreynska *et al.*, J. Bact. 176:5938, 1994); a 30 kDa outer membrane protein (Bölin *et al.*, J. Clin. Microbiol. 33:381, 1995); a lactoferrin receptor (FR 2,724,936); and several porins, designated HopA, HopB, HopC, HopD, and HopE, which have molecular weights of 48-67 kDa (Exner *et al.*, Infect. Immun. 63:1567, 1995; Doig *et al.*, J. Bact. 177:5447, 1995). Some of these proteins have been proposed as potential vaccine antigens. In particular, urease is believed to be a vaccine candidate (WO 94/9823; WO 95/22987; WO 95/3824; Michetti *et al.*, Gastroenterology 107:1002, 1994). Nevertheless, it is thought that several antigens may ultimately be necessary in a vaccine.

Summary of the Invention

20 The invention provides polynucleotide molecules that encode a family of 76 kDa *Helicobacter* polypeptides, designated GHPO 386, GHPO 789, GHPO 1516, GHPO 1197, GHPO 1180, GHPO 896, GHPO 711, GHPO 190, GHPO 185, GHPO 1417, and GHPO 1414, a 32 kDa polypeptide, designated GHPO 1360, and a 50 kDa polypeptide, designated GHPO 750, which can be used, *e.g.*, in methods to prevent, treat, or diagnose *Helicobacter* infection. The polypeptides include those having the amino acid sequences

shown in SEQ ID NOs:2-22 (even numbers), 66, and 68. Those skilled in the art will understand that the invention also includes polynucleotide molecules that encode mutants and derivatives of these polypeptides, which can result from the addition, deletion, or substitution of non-essential amino acids, as is described further below.

In addition to the polynucleotide molecules described above, the invention includes the corresponding polypeptides (*i.e.*, polypeptides encoded by the polynucleotide molecules of the invention, or fragments thereof), and monospecific antibodies that specifically bind to these polypeptides.

The present invention has many applications and includes expression cassettes, vectors, and cells transformed or transfected with the polynucleotides of the invention. Accordingly, the present invention provides (i) methods for producing polypeptides of the invention in recombinant host systems and related expression cassettes, vectors, and transformed or transfected cells; (ii) live vaccine vectors, such as pox virus, *Salmonella typhimurium*, and *Vibrio cholerae* vectors, that contain polynucleotides of the invention (such vaccine vectors being useful in, *e.g.*, methods for preventing or treating *Helicobacter* infection) in combination with a diluent or carrier, and related pharmaceutical compositions and associated therapeutic and/or prophylactic methods; (iii) therapeutic and/or prophylactic methods involving administration of polynucleotide molecules, either in a naked form or formulated with a delivery vehicle, polypeptides or mixtures of polypeptides, or monospecific antibodies of the invention, and related pharmaceutical compositions; (iv) methods for detecting the presence of *Helicobacter* in biological samples, which can involve the use of polynucleotide molecules, monospecific antibodies, or polypeptides of the invention; and (v) methods for purifying polypeptides of the invention by antibody-based affinity chromatography.

Brief Description of the Drawings

Figure 1 is an alignment of the predicted amino acid sequences of GHPO 386 (SEQ ID NO:2), GHPO 789 (SEQ ID NO:4), and GHPO 1516 (SEQ ID NO:6), as well as a consensus sequence for the 76 kDa protein family.

5 Figure 2 is an alignment of the predicted amino acid sequences of GHPO 1197 (SEQ ID NO:8), GHPO 1180 (SEQ ID NO:10), GHPO 896 (SEQ ID NO:12), GHPO 711 (SEQ ID NO:14), GHPO 190 (SEQ ID NO:16), GHPO 185 (SEQ ID NO:18), GHPO 1417 (SEQ ID NO:20), and GHPO 1414 (SEQ ID NO:22), as well as a consensus sequence for the 76 kDa protein family.

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Detailed Description

Open reading frames (ORFs) encoding a family of new, full length, membrane-associated 76 kDa polypeptides, designated GHPO 386, GHPO 789, GHPO 1516, GHPO 1197, GHPO 1180, GHPO 896, GHPO 711, GHPO 190, GHPO 185, GHPO 1417, and GHPO 1414, a 32 kDa polypeptide, designated
15 GHPO 1360, and a 50 kDa polypeptide, designated GHPO 750, have been identified in the *H. pylori* genome. The amino acid sequences of the 76 kDa polypeptides are aligned in Figures 1 and 2. The 76 kDa, 32 kDa, and 50 kDa polypeptides can be used, for example, in vaccination methods for preventing or treating *Helicobacter* infection. For example, GHPO 750, GHPO 1360,
20 GHPO 190, and GHPO 1516 have been shown to be protective antigens. By “protective antigen” is meant an antigen that is capable of reducing the infection level after challenge, relative to a positive control. Absolute protection from infection, although included in the invention, is not required.

The polypeptides of the invention (except GHPO 750, see below) are
25 secreted polypeptides that can be produced in their mature forms (*i.e.*, as polypeptides that have been exported through class II or class III secretion

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pathways) or as precursors that include a signal peptide, which can be removed in the course of excretion/secretion by cleavage at the N-terminal end of the mature form. (The cleavage site is located at the C-terminal end of the signal peptide, adjacent to the mature form.) The cleavage site for the polypeptides of the invention and, thus, the first amino acid of the mature polypeptides, was putatively determined.

According to a first aspect of the invention, there are provided isolated polynucleotides that encode the precursor and mature forms of *Helicobacter* GHPO 386, GHPO 789, GHPO 1516, GHPO 1197, GHPO 1180, GHPO 896, GHPO 711, GHPO 190, GHPO 185, GHPO 1417, GHPO 1414, GHPO 1360, and GHPO 750.

An isolated polynucleotide of the invention encodes:

(i) a polypeptide having an amino acid sequence that is homologous to a *Helicobacter* amino acid sequence of a polypeptide associated with the *Helicobacter* membrane, the *Helicobacter* amino acid sequence being selected from the group consisting of the amino acid sequences shown:

-in SEQ ID NO:2, beginning with an amino acid in any one of positions -19 to 5, preferably in position -19 or position 1, and ending with an amino acid in position 689 (GHPO 386);

-in SEQ ID NO:4, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 713 (GHPO 789);

-in SEQ ID NO:6, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 725 (GHPO 1516);

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-in SEQ ID NO:8, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 691 (GHPO 1197);

5 -in SEQ ID NO:10, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 652 (GHPO 1180);

-in SEQ ID NO:12, beginning with an amino acid in any one of positions -18 to 5, preferably in position -18 or position 1, and ending with an amino acid in position 673 (GHPO 896);

10 -in SEQ ID NO:14, beginning with an amino acid in any one of positions -21 to 5, preferably in position -21 or position 1, and ending with an amino acid in position 619 (GHPO 711);

15 -in SEQ ID NO:16, beginning with an amino acid in any one of positions -17 to 5, preferably in position -17 or position 1, and ending with an amino acid in position 635 (GHPO 190);

-in SEQ ID NO:18, beginning with an amino acid in any one of positions -19 to 5, preferably in position -19 or position 1, and ending with an amino acid in position 626 (GHPO 185);

20 -in SEQ ID NO:20, beginning with an amino acid in any one of positions -16 to 5, preferably in position -16 or position 1, and ending with an amino acid in position 467 (GHPO 1417);

-in SEQ ID NO:22, beginning with an amino acid in any one of positions -18 to 5, preferably in position -18 or position 1, and ending with an amino acid in position 673 (GHPO 1414);

25 -in SEQ ID NO:66, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 279 (GHPO 1360); and

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-in SEQ ID NO:68, beginning with an amino acid in position 1 and ending with an amino acid in position 399 (GHPO 750); or

(ii) a derivative of the polypeptide.

The term "isolated polynucleotide" is defined as a polynucleotide
5 that is removed from the environment in which it naturally occurs. For example, a naturally-occurring DNA molecule present in the genome of a living bacteria or as part of a gene bank is not isolated, but the same molecule, separated from the remaining part of the bacterial genome, as a result of, *e.g.*, a cloning event (amplification), is "isolated." Typically, an isolated DNA
10 molecule is free from DNA regions (*e.g.*, coding regions) with which it is immediately contiguous, at the 5' or 3' ends, in the naturally occurring genome. Such isolated polynucleotides can be part of a vector or a composition and still be isolated, as such a vector or composition is not part of its natural environment.

15 A polynucleotide of the invention can consist of RNA or DNA (*e.g.*, cDNA, genomic DNA, or synthetic DNA), or modifications or combinations of RNA or DNA. The polynucleotide can be double-stranded or single-stranded and, if single-stranded, can be the coding (sense) strand or the non-coding (anti-sense) strand. The sequences that encode polypeptides of the invention, as
20 shown in SEQ ID NOs:2-22 (even numbers), 66, and 68, can be (a) the coding sequence as shown in SEQ ID NOs:1-21 (odd numbers), 65, and 67; (b) a ribonucleotide sequence derived by transcription of (a); or (c) a different coding sequence that, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptides as the polynucleotide molecules having
25 the sequences illustrated in any of SEQ ID NOs:1-21 (odd numbers), 65, and 67. The polypeptides of the invention can be ones that are naturally secreted or excreted by, *e.g.*, *H. felis*, *H. mustelae*, *H. heilmanii*, or *H. pylori*.

By "polypeptide" or "protein" is meant any chain of amino acids, regardless of length or post-translational modification (*e.g.*, glycosylation or phosphorylation). Both terms are used interchangeably in the present application.

5 By "homologous amino acid sequence" is meant an amino acid sequence that differs from an amino acid sequence shown in any of SEQ ID NOs:2-22 (even numbers), 66, and 68, or an amino acid sequence encoded by the nucleotide sequence of any of SEQ ID NOs:1-21 (odd numbers), 65, and 67, by one or more non-conservative amino acid substitutions, deletions, or
10 additions located at positions at which they do not destroy the specific antigenicity of the polypeptide. Preferably, such a sequence is at least 75%, more preferably at least 80%, and most preferably at least 90% identical to an amino acid sequence shown in any of SEQ ID NOs:2-22 (even numbers), 66, and 68.

15 Homologous amino acid sequences include sequences that are identical or substantially identical to an amino acid sequence as shown in any of SEQ ID NOs:2-22 (even numbers), 66, and 68. By "amino acid sequence that is substantially identical" is meant a sequence that is at least 90%, preferably at least 95%, more preferably at least 97%, and most preferably at
20 least 99% identical to an amino acid sequence of reference and that differs from the sequence of reference, if at all, by a majority of conservative amino acid substitutions.

Conservative amino acid substitutions typically include substitutions among amino acids of the same class. These classes include, for example,
25 amino acids having uncharged polar side chains, such as asparagine, glutamine, serine, threonine, and tyrosine; amino acids having basic side chains, such as lysine, arginine, and histidine; amino acids having acidic side chains, such as

aspartic acid and glutamic acid; and amino acids having nonpolar side chains, such as glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and cysteine.

Homology can be measured using sequence analysis software (*e.g.*,
5 Sequence Analysis Software Package of the Genetics Computer Group,
University of Wisconsin Biotechnology Center, 1710 University Avenue,
Madison, WI 53705). Similar amino acid sequences are aligned to obtain the
maximum degree of homology (*i.e.*, identity). To this end, it may be necessary
to artificially introduce gaps into the sequence. Once the optimal alignment has
10 been set up, the degree of homology (*i.e.*, identity) is established by recording
all of the positions in which the amino acids of both sequences are identical,
relative to the total number of positions.

Homologous polynucleotide sequences are defined in a similar way.
Preferably, a homologous sequence is one that is at least 45%, more preferably
15 at least 60%, and most preferably at least 85% identical to a coding sequence of
any of SEQ ID NOs:1-21 (odd numbers), 65, and 67.

Polypeptides having a sequence homologous to one of the sequences
shown in SEQ ID NOs:2-22 (even numbers), 66, and 68 include naturally-
occurring allelic variants, as well as mutants or any other non-naturally
20 occurring variants that are analogous in terms of antigenicity, to a polypeptide
having a sequence as shown in SEQ ID NOs:2-22 (even numbers), 66, and 68.

As is known in the art, an allelic variant is an alternate form of a
polypeptide that is characterized as having a substitution, deletion, or addition
of one or more amino acids that does not alter the biological function of the
25 polypeptide. By "biological function" is meant a function of the polypeptide in
the cells in which it naturally occurs, even if the function is not necessary for
the growth or survival of the cells. For example, the biological function of a

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porin is to allow the entry into cells of compounds present in the extracellular medium. The biological function is distinct from the antigenic function. A polypeptide can have more than one biological function.

Allelic variants are very common in nature. For example, a bacterial species, *e.g.*, *H. pylori*, is usually represented by a variety of strains that differ from each other by minor allelic variations. Indeed, a polypeptide that fulfills the same biological function in different strains can have an amino acid sequence that is not identical in each of the strains. Such an allelic variation can be equally reflected at the polynucleotide level.

Support for the use of allelic variants of polypeptide antigens comes from, *e.g.*, studies of the *Helicobacter* urease antigen. The amino acid sequence of *Helicobacter* urease varies widely from species to species, yet cross-species protection occurs, indicating that the urease molecule, when used as an immunogen, is highly tolerant of amino acid variations. Even among different strains of the single species *H. pylori*, there are amino acid sequence variations.

For example, although the amino acid sequences of the UreA and UreB subunits of *H. pylori* and *H. felis* ureases differ from one another by 26.5% and 11.8%, respectively (Ferrero *et al.*, Molecular Microbiology 9(2):323-333, 1993), it has been shown that *H. pylori* urease protects mice from *H. felis* infection (Michetti *et al.*, Gastroenterology 107:1002, 1994). In addition, it has been shown that the individual structural subunits of urease, UreA and UreB, which contain distinct amino acid sequences, are both protective antigens against *Helicobacter* infection (Michetti *et al.*, *supra*): Similarly, Cuenca *et al.* (Gastroenterology 110:1770, 1996) showed that therapeutic immunization of *H. mustelae*-infected ferrets with *H. pylori* urease was effective at eradicating *H. mustelae* infection. Further, several urease

variants have been reported to be effective vaccine antigens, including, *e.g.*, recombinant UreA + UreB apoenzyme expressed from pORV142 (UreA and UreB sequences derived from *H. pylori* strain CPM630; Lee *et al.*, J. Infect. Dis. 172:161, 1995); recombinant UreA + UreB apoenzyme expressed from
5 pORV214 (UreA and UreB sequences differ from *H. pylori* strain CPM630 by one and two amino acid changes, respectively; Lee *et al.*, *supra*, 1995); a UreA-glutathione-S-transferase fusion protein (UreA sequence from *H. pylori* strain ATCC 43504; Thomas *et al.*, Acta Gastro-Enterologica Belgica 56:54, 1993); UreA + UreB holoenzyme purified from *H. pylori* strain NCTC11637
10 (Marchetti *et al.*, Science 267:1655, 1995); a UreA-MBP fusion protein (UreA from *H. pylori* strain 85P; Ferrero *et al.*, Infection and Immunity 62:4981, 1994); a UreB-MBP fusion protein (UreB from *H. pylori* strain 85P; Ferrero *et al.*, *supra*); a UreA-MBP fusion protein (UreA from *H. felis* strain ATCC 49179; Ferrero *et al.*, *supra*); a UreB-MBP fusion protein (UreB from *H. felis*
15 strain ATCC 49179; Ferrero *et al.*, *supra*); and a 37 kDa fragment of UreB containing amino acids 220-569 (Dore-Davin *et al.*, "A 37 kD fragment of UreB is sufficient to confer protection against *Helicobacter felis* infection in mice"). Finally, Thomas *et al.* (*supra*) showed that oral immunization of mice with crude sonicates of *H. pylori* protected mice from subsequent challenge
20 with *H. felis*.

Polynucleotides, *e.g.*, DNA molecules, encoding allelic variants can easily be obtained by polymerase chain reaction (PCR) amplification of genomic bacterial DNA extracted by conventional methods. This involves the use of synthetic oligonucleotide primers matching sequences that are upstream
25 and downstream of the 5' and 3' ends of the coding region. Suitable primers can be designed based on the nucleotide sequence information provided in SEQ ID NOs:1-21 (odd numbers), 65, and 67. Typically, a primer consists of 10 to

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40, preferably 15 to 25 nucleotides. It can also be advantageous to select primers containing C and G nucleotides in proportions sufficient to ensure efficient hybridization, e.g., an amount of C and G nucleotides of at least 40%, preferably 50%, of the total nucleotide amount. Those skilled in the art can readily design primers that can be used to isolate the polynucleotides of the invention from different *Helicobacter* strains.

As an example, primers useful for cloning a polynucleotide molecule encoding a polypeptide having the amino acid sequence of unprocessed GHPO 386 (SEQ ID NO:2), including a signal peptide, are shown in SEQ ID NO:23 (matching at the 5' end) and in SEQ ID NO:25 (matching at the 3' end). Primers useful for cloning a DNA molecule encoding a polypeptide having the amino acid sequence of mature GHPO 386 (amino acids 1-689 of SEQ ID NO:2), lacking a signal peptide, are shown in SEQ ID NO:24 (matching at the 5' end) and in SEQ ID NO:25 (matching at the 3' end). Primers useful for cloning a DNA molecule encoding a polypeptide having the amino acid sequence of GHPO 1360 (SEQ ID NO:66), are shown in SEQ ID NO:78 (matching at the 5' end) and in SEQ ID NO:79 (matching at the 3' end). Use of these primers enables amplification of the entire gene encoding GHPO 1360. Primers having sequences shown in SEQ ID NO:82 (matching at the 5' end of the coding sequence corresponding to the mature protein) and SEQ ID NO:79 (matching at the 3' end) can be used to amplify the portion of the gene encoding mature GHPO 1360. Experimental conditions for carrying out PCR can readily be determined by one skilled in the art and illustrations of carrying out PCR are provided in Examples 3 and 4.

Thus, the first aspect of the invention includes:

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(i) isolated polynucleotide molecules (e.g., DNA molecules) that can be amplified and/or cloned using the polymerase chain reaction from a *Helicobacter*, e.g., *H. pylori*, genome using either:

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:23, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:25 (unprocessed GHPO 386);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:26, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:28 (unprocessed GHPO 789);
- 10 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:29, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:31 (unprocessed GHPO 1516);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:32, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:34 (unprocessed GHPO 1197);
- 15 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:35, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:37 (unprocessed GHPO 1180);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:38, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:40 (unprocessed GHPO 896);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:41, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:43 (unprocessed GHPO 711);
- 20 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:44, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:46 (unprocessed GHPO 190);

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- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:47, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:49 (unprocessed GHPO 185);

5 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:50, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:52 (unprocessed GHPO 1417);

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:53, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:55 (unprocessed GHPO 1414);

10 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:78, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:79 (unprocessed GHPO 1360); or

15 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:80, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:81 (GHPO 750); and

(ii) isolated polynucleotide molecules (*e.g.*, DNA molecules) that can be amplified and/or cloned by the polymerase chain reaction from a *Helicobacter*, *e.g.*, *H. pylori*, genome using either:

20 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:24, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:25 (mature GHPO 386);

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:27, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:28 (mature GHPO 789);

25 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:30, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:31 (mature GHPO 1516);

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- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:33, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:34 (mature GHPO 1197);

5 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:36, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:37 (mature GHPO 1180);

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:39, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:40 (mature GHPO 896);

10 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:42, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:43 (mature GHPO 711);

15 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:45, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:46 (mature GHPO 190);

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:48, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:49 (mature GHPO 185);

20 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:51, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:52 (mature GHPO 1417);

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:54, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:55 (mature GHPO 1414); or

25 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:82, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:79 (mature GHPO 1360).

The 5' ends of the primers described above can advantageously include a restriction endonuclease recognition site that contains, typically, 4 to 6 nucleotides. For example, the sequences 5'-GGATCC-3' (*Bam*HI) or 5'-CTCGAG-3' (*Xho*I) can be used. Restriction sites can be selected by those skilled in the art so that the amplified DNA, when digested, if necessary, can be conveniently cloned into an appropriately digested vector, such as a plasmid vector. In addition, a 5' clamp (*e.g.*, GCC) can be included in the primers 5' to the restriction endonuclease recognition site.

Useful homologs that do not occur naturally can be designed using known methods for identifying regions of an antigen that are likely to be tolerant of amino acid sequence changes and/or deletions. For example, sequences of the antigen from different species can be compared to identify conserved sequences.

Polypeptide derivatives that are encoded by polynucleotides of the invention include, *e.g.*, fragments, polypeptides having large internal deletions derived from full-length polypeptides, and fusion proteins. Polypeptide fragments of the invention can be derived from a polypeptide having a sequence homologous to the sequences of any of SEQ ID NOs:2-22 (even numbers), 66, and 68, to the extent that the fragments retain the substantial antigenicity of the parent polypeptide (specific antigenicity). Polypeptide derivatives can also be constructed by large internal deletions that remove a substantial part of the parent polypeptide, while retaining specific antigenicity. Generally, polypeptide derivatives should be about at least 12 amino acids in length to maintain antigenicity. Advantageously, they can be at least 20 amino acids, preferably at least 50 amino acids, more preferably at least 75 amino acids, and most preferably at least 100 amino acids in length.

Useful polypeptide derivatives, *e.g.*, polypeptide fragments, can be designed using computer-assisted analysis of amino acid sequences in order to identify sites in protein antigens having potential as surface-exposed, antigenic regions (Hughes *et al.*, Infect. Immun. 60(9):3497, 1992). For example, the
5 Laser Gene Program from DNA Star can be used to obtain hydrophilicity, antigenic index, and intensity index plots for the polypeptides of the invention. This program can also be used to obtain information about homologies of the polypeptides with known protein motifs. One skilled in the art can readily use the information provided in such plots to select peptide fragments for use as
10 vaccine antigens. For example, fragments spanning regions of the plots in which the antigenic index is relatively high can be selected. One can also select fragments spanning regions in which both the antigenic index and the intensity plots are relatively high. Fragments containing conserved sequences, particularly hydrophilic conserved sequences, can also be selected.

15 Polypeptide fragments and polypeptides having large internal deletions can be used for revealing epitopes that are otherwise masked in the parent polypeptide and that may be of importance for inducing a protective T cell-dependent immune response. Deletions can also remove immunodominant regions of high variability among strains.

20 It is an accepted practice in the field of immunology to use fragments and variants of protein immunogens as vaccines, as all that is required to induce an immune response to a protein is a small (*e.g.*, 8 to 10 amino acids) immunogenic region of the protein. This has been done for a number of vaccines against pathogens other than *Helicobacter*. For example, short
25 synthetic peptides corresponding to surface-exposed antigens of pathogens such as murine mammary tumor virus (peptide containing 11 amino acids; Dion *et al.*, Virology 179:474-477, 1990), Semliki Forest virus (peptide containing 16

amino acids; Snijders *et al.*, J. Gen. Virol. 72:557-565, 1991), and canine parvovirus (2 overlapping peptides, each containing 15 amino acids; Langeveld *et al.*, Vaccine 12(15):1473-1480, 1994) have been shown to be effective vaccine antigens against their respective pathogens.

5 Polynucleotides encoding polypeptide fragments and polypeptides having large internal deletions can be constructed using standard methods (see, *e.g.*, Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons Inc., 1994), for example, by PCR, including inverse PCR, by restriction enzyme treatment of the cloned DNA molecules, or by the method of Kunkel *et al.* (Proc. Natl. Acad. Sci. USA 82:448, 1985; biological material available at
10 Stratagene).

A polypeptide derivative can also be produced as a fusion polypeptide that contains a polypeptide or a polypeptide derivative of the invention fused, *e.g.*, at the – or C-terminal end, to any other polypeptide
15 (hereinafter referred to as a peptide tail). Such a product can be easily obtained by translation of a genetic fusion, *i.e.*, a hybrid gene. Vectors for expressing fusion polypeptides are commercially available, and include the pMal-c2 or pMal-p2 systems of New England Biolabs, in which the peptide tail is a maltose binding protein, the glutathione-S-transferase system of Pharmacia, or
20 the His-Tag system available from Novagen. These and other expression systems provide convenient means for further purification of polypeptides and derivatives of the invention.

Another particular example of fusion polypeptides included in invention includes a polypeptide or polypeptide derivative of the invention
25 fused to a polypeptide having adjuvant activity, such as, *e.g.*, subunit B of either cholera toxin or *E. coli* heat-labile toxin. Several possibilities can be

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used for producing such fusion proteins. First, the polypeptide of the invention can be fused to the

N-terminal end or, preferably, to the C-terminal end of the polypeptide having adjuvant activity. Second, a polypeptide fragment of the invention can be fused
5 within the amino acid sequence of the polypeptide having adjuvant activity.

Spacer sequences can also be included, if desired.

As stated above, the polynucleotides of the invention encode *Helicobacter* polypeptides in precursor or mature form. They can also encode hybrid precursors containing heterologous signal peptides, which can mature
10 into polypeptides of the invention. By "heterologous signal peptide" is meant a signal peptide that is not found in the naturally-occurring precursor of a polypeptide of the invention.

A polynucleotide of the invention hybridizes, preferably under stringent conditions, to a polynucleotide having a sequence as shown in any of
15 SEQ ID NOs:1-21 (odd numbers), 65, and 67. Hybridization procedures are, e.g., described by Ausubel *et al.* (*supra*); Silhavy *et al.* (*Experiments with Gene Fusions*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1984); and Davis *et al.* (*A Manual for Genetic Engineering: Advanced Bacterial Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor,
20 New York, 1980). Important parameters that can be considered for optimizing hybridization conditions are reflected in the following formula, which facilitates calculation of the melting temperature (T_m), which is the temperature above which two complementary DNA strands separate from one another (Casey *et al.*, Nucl. Acid Res. 4:1539, 1977): $T_m = 81.5 + 0.5 \cdot x - (\% G+C) + 1.6 \log (\text{positive ion concentration}) - 0.6 \cdot x (\% \text{ formamide})$. Under
25 appropriate stringency conditions, hybridization temperature (T_h) is approximately 20 to 40°C, 20 to 25°C, or, preferably, 30 to 40°C below the

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calculated T_m . Those skilled in the art will understand that optimal temperature and salt conditions can be readily determined empirically in preliminary experiments using conventional procedures. For example, stringent conditions can be achieved, both for pre-hybridizing and hybridizing incubations, (i) within 4-16 hours at 42°C, in 6 x SSC containing 50% formamide or (ii) within 4-16 hours at 65°C in an aqueous 6 x SSC solution (1 M NaCl, 0.1 M sodium citrate (pH 7.0)). For polynucleotides containing 30 to 600 nucleotides, the above formula is used and then is corrected by subtracting (600/polynucleotide size in base pairs). Stringency conditions are defined by a T_h that is 5 to 10°C below T_m .

Hybridization conditions with oligonucleotides shorter than 20-30 bases do not precisely follow the rules set forth above. In such cases, the formula for calculating the T_m is as follows: $T_m = 4 \times (G+C) + 2 \times (A+T)$. For example, an 18 nucleotide fragment of 50% G+C would have an approximate T_m of 54°C.

A polynucleotide molecule of the invention, containing RNA, DNA, or modifications or combinations thereof, can have various applications. For example, a polynucleotide molecule can be used (i) in a process for producing the encoded polypeptide in a recombinant host system, (ii) in the construction of vaccine vectors such as poxviruses, which are further used in methods and compositions for preventing and/or treating *Helicobacter* infection, (iii) as a vaccine agent, in a naked form or formulated with a delivery vehicle, and (iv) in the construction of attenuated *Helicobacter* strains that can over-express a polynucleotide of the invention or express it in a non-toxic, mutated form.

According to a second aspect of the invention, there is therefore provided (i) an expression cassette containing a polynucleotide molecule of the invention placed under the control of elements (e.g., a promoter) required for

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expression; (ii) an expression vector containing an expression cassette of the invention; (iii) a procaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention; as well as (iv) a process for producing a polypeptide or polypeptide derivative encoded by a

5 polynucleotide of the invention, which involves culturing a procaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, under conditions that allow expression of the polynucleotide molecule of the invention and, recovering the encoded polypeptide or polypeptide derivative from the cell culture.

10 A recombinant expression system can be selected from procaryotic and eucaryotic hosts. Eucaryotic hosts include, for example, yeast cells (*e.g.*, *Saccharomyces cerevisiae* or *Pichia pastoris*), mammalian cells (*e.g.*, COS1, NIH3T3, or JEG3 cells), arthropods cells (*e.g.*, *Spodoptera frugiperda* (SF9) cells), and plant cells. Preferably, a procaryotic host such as *E. coli* is used.

15 Bacterial and eucaryotic cells are available from a number of different sources that are known to those skilled in the art, *e.g.*, the American Type Culture Collection (ATCC; Rockville, Maryland).

The choice of the expression cassette will depend on the host system selected, as well as the features desired for the expressed polypeptide. For
20 example, it may be useful to produce a polypeptide of the invention in a particular lipidated form or any other form. Typically, an expression cassette includes a constitutive or inducible promoter that is functional in the selected host system; a ribosome binding site; a start codon (ATG); if necessary, a region encoding a signal peptide, *e.g.*, a lipidation signal peptide; a
25 polynucleotide molecule of the invention; a stop codon; and, optionally, a 3' terminal region (translation and/or transcription terminator). The signal peptide-encoding region is adjacent to the polynucleotide of the invention and

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is placed in the proper reading frame. The signal peptide-encoding region can be homologous or heterologous to the polynucleotide molecule encoding the mature polypeptide and it can be specific to the secretion apparatus of the host used for expression. The open reading frame constituted by the polynucleotide molecule of the invention, alone or together with the signal peptide, is placed under the control of the promoter so that transcription and translation occur in the host system. Promoters and signal peptide-encoding regions are widely known and available to those skilled in the art and include, for example, the promoter of *Salmonella typhimurium* (and derivatives) that is inducible by arabinose (promoter araB) and is functional in Gram-negative bacteria such as *E. coli* (U.S. Patent No. 5,028,530; Cagnon *et al.*, Protein Engineering 4(7):843, 1991); the promoter of the bacteriophage T7 RNA polymerase gene, which is functional in a number of *E. coli* strains expressing T7 polymerase (U.S. Patent No. 4,952,496); the OspA lipidation signal peptide; and RlpB lipidation signal peptide (Takase *et al.*, J. Bact. 169:5692, 1987).

The expression cassette is typically part of an expression vector, which is selected for its ability to replicate in the chosen expression system. Expression vectors (*e.g.*, plasmids or viral vectors) can be chosen from, for example, those described in Pouwels *et al.* (*Cloning Vectors: A Laboratory Manual*, 1985, Supp. 1987) and can be purchased from various commercial sources. Methods for transforming or transfecting host cells with expression vectors are well known in the art and will depend on the host system selected, as described in Ausubel *et al.* (*supra*).

Upon expression, a recombinant polypeptide of the invention (or a polypeptide derivative) is produced and remains in the intracellular compartment, is secreted/excreted in the extracellular medium or in the periplasmic space, or is embedded in the cellular membrane. The polypeptide

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can then be recovered in a substantially purified form from the cell extract or from the supernatant after centrifugation of the cell culture. Typically, the recombinant polypeptide can be purified by antibody-based affinity purification or by any other method known to a person skilled in the art, such as by genetic fusion to a small affinity-binding domain. Antibody-based affinity purification methods are also available for purifying a polypeptide of the invention extracted from a *Helicobacter* strain. Antibodies useful for immunoaffinity purification of the polypeptides of the invention can be obtained using methods described below.

Polynucleotides of the invention can also be used in DNA vaccination methods, using either a viral or bacterial host as gene delivery vehicle (live vaccine vector) or administering the gene in a free form, *e.g.*, inserted into a plasmid. Therapeutic or prophylactic efficacy of a polynucleotide of the invention can be evaluated as is described below.

Accordingly, in a third aspect of the invention, there is provided (i) a vaccine vector such as a poxvirus, containing a polynucleotide molecule of the invention placed under the control of elements required for expression; (ii) a composition of matter containing a vaccine vector of the invention, together with a diluent or carrier; (iii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a vaccine vector of the invention; (iv) a method for inducing an immune response against *Helicobacter* in a mammal (*e.g.*, a human; alternatively, the method can be used in veterinary applications for treating or preventing *Helicobacter* infection of animals, *e.g.*, cats or birds), which involves administering to the mammal an immunogenically effective amount of a vaccine vector of the invention to elicit an immune response, *e.g.*, a protective or therapeutic immune response to *Helicobacter*; and (v) a method for preventing and/or treating a *Helicobacter*

(e.g., *H. pylori*, *H. felis*, *H. mustelae*, or *H. heilmanii*) infection, which involves administering a prophylactic or therapeutic amount of a vaccine vector of the invention to an individual in need. Additionally, the third aspect of the invention encompasses the use of a vaccine vector of the invention in the
5 preparation of a medicament for preventing and/or treating *Helicobacter* infection.

A vaccine vector of the invention can express one or several polypeptides or derivatives of the invention, as well as at least one additional *Helicobacter* antigen such as a urease apoenzyme or a subunit, fragment,
10 homolog, mutant, or derivative thereof. In addition, it can express a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12), that enhances the immune response. Thus, a vaccine vector can include an additional polynucleotide molecules encoding, e.g., urease subunit A, B, or both, or a cytokine, placed under the control of elements required for expression in a
15 mammalian cell.

Alternatively, a composition of the invention can include several vaccine vectors, each of which being capable of expressing a polypeptide or derivative of the invention. A composition can also contain a vaccine vector capable of expressing an additional *Helicobacter* antigen such as urease
20 apoenzyme, a subunit, fragment, homolog, mutant, or derivative thereof, or a cytokine such as IL-2 or IL-12.

In vaccination methods for treating or preventing infection in a mammal, a vaccine vector of the invention can be administered by any conventional route in use in the vaccine field, for example, to a mucosal (e.g.,
25 ocular, intranasal, oral, gastric, pulmonary, intestinal, rectal, vaginal, or urinary tract) surface or *via* a parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. Preferred routes depend

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upon the choice of the vaccine vector. The administration can be achieved in a single dose or repeated at intervals. The appropriate dosage depends on various parameters that are understood by those skilled in the art, such as the nature of the vaccine vector itself, the route of administration, and the condition of the mammal to be vaccinated (*e.g.*, the weight, age, and general health of the mammal).

Live vaccine vectors that can be used in the invention include viral vectors, such as adenoviruses and poxviruses, as well as bacterial vectors, *e.g.*, *Shigella*, *Salmonella*, *Vibrio cholerae*, *Lactobacillus*, Bacille bilié de Calmette-Guérin (BCG), and *Streptococcus*. An example of an adenovirus vector, as well as a method for constructing an adenovirus vector capable of expressing a polynucleotide molecule of the invention, is described in U.S. Patent No. 4,920,209. Poxvirus vectors that can be used in the invention include, *e.g.*, vaccinia and canary pox viruses, which are described in U.S. Patent No. 4,722,848 and U.S. Patent No. 5,364,773, respectively (also see, *e.g.*, Tartaglia *et al.*, Virology 188:217, 1992, for a description of a vaccinia virus vector, and Taylor *et al.*, Vaccine 13:539, 1995, for a description of a canary poxvirus vector). Poxvirus vectors capable of expressing a polynucleotide of the invention can be obtained by homologous recombination, as described in Kieny *et al.* (Nature 312:163, 1984) so that the polynucleotide of the invention is inserted in the viral genome under appropriate conditions for expression in mammalian cells. Generally, the dose of viral vector vaccine, for therapeutic or prophylactic use, can be from about 1×10^4 to about 1×10^{11} , advantageously from about 1×10^7 to about 1×10^{10} , or, preferably, from about 1×10^7 to about 1×10^9 plaque-forming units per kilogram. Preferably, viral vectors are administered parenterally, for example, in 3 doses that are 4 weeks apart. Those skilled in the art will recognize that it is preferable to avoid adding a

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chemical adjuvant to a composition containing a viral vector of the invention and thereby minimizing the immune response to the viral vector itself.

Non-toxicogenic *Vibrio cholerae* mutant strains that can be used in live oral vaccines are described by Mekalanos *et al.* (Nature 306:551, 1983) and in U.S. Patent No. 4,882,278 (strain in which a substantial amount of the coding sequence of each of the two *ctxA* alleles has been deleted so that no functional *cholerae* toxin is produced); WO 92/11354 (strain in which the *irgA* locus is inactivated by mutation; this mutation can be combined in a single strain with *ctxA* mutations); and WO 94/1533 (deletion mutant lacking functional *ctxA* and *attRSI* DNA sequences). These strains can be genetically engineered to express heterologous antigens, as described in WO 94/19482. An effective vaccine dose of a *V. cholerae* strain capable of expressing a polypeptide or polypeptide derivative encoded by a polynucleotide molecule of the invention can contain, *e.g.*, about 1×10^5 to about 1×10^9 , preferably about 1×10^6 to about 1×10^8 viable bacteria in an appropriate volume for the selected route of administration. Preferred routes of administration include all mucosal routes, but, most preferably, these vectors are administered intranasally or orally.

Attenuated *Salmonella typhimurium* strains, genetically engineered for recombinant expression of heterologous antigens, and their use as oral vaccines, are described by Nakayama *et al.* (Bio/Technology 6:693, 1988) and in WO 92/11361. Preferred routes of administration for these vectors include all mucosal routes. Most preferably, the vectors are administered intranasally or orally.

Others bacterial strains useful as vaccine vectors are described by High *et al.* (EMBO 11:1991, 1992) and Sizemore *et al.* (Science 270:299, 1995; *Shigella flexneri*); Medaglini *et al.* (Proc. Natl. Acad. Sci. USA 92:6868,

1995; (*Streptococcus gordonii*); Flynn (Cell. Mol. Biol. 40 (suppl. I):31, 1194), and in WO 88/6626, WO 90/0594, WO 91/13157, WO 92/1796, and WO 92/21376 (Bacille Calmette Guerin). In bacterial vectors, a polynucleotide of the invention can be inserted into the bacterial genome or it can remain in a free state, for example, carried on a plasmid.

An adjuvant can also be added to a composition containing a bacterial vector vaccine. A number of adjuvants that can be used are known to those skilled in the art. For example, preferred adjuvants can be selected from the list provided below.

10 According to a fourth aspect of the invention, there is also provided (i) a composition of matter containing a polynucleotide of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a polynucleotide of the invention; (iii) a method for inducing an immune response against
15 *Helicobacter*, in a mammal, by administering to the mammal an immunogenically effective amount of a polynucleotide of the invention to elicit an immune response, *e.g.*, a protective immune response to *Helicobacter*; and (iv) a method for preventing and/or treating a *Helicobacter* (*e.g.*, *H. pylori*, *H. felis*, *H. mustelae*, or *H. heilmanii*) infection, by administering a prophylactic or
20 therapeutic amount of a polynucleotide of the invention to an individual in need of such treatment. Additionally, the fourth aspect of the invention encompasses the use of a polynucleotide of the invention in the preparation of a medicament for preventing and/or treating *Helicobacter* infection. The fourth aspect of the invention preferably includes the use of a polynucleotide molecule placed
25 under conditions for expression in a mammalian cell, *e.g.*, in a plasmid that is unable to replicate in mammalian cells and to substantially integrate into a mammalian genome.

Polynucleotides (for example, DNA or RNA molecules) of the invention can also be administered as such to a mammal as a vaccine. When a DNA molecule of the invention is used, it can be in the form of a plasmid that is unable to replicate in a mammalian cell and unable to integrate into the mammalian genome. Typically, a DNA molecule is placed under the control of a promoter suitable for expression in a mammalian cell. The promoter can function ubiquitously or tissue-specifically. Examples of non-tissue specific promoters include the early Cytomegalovirus (CMV) promoter (U.S. Patent No. 4,168,062) and the Rous Sarcoma Virus promoter (Norton *et al.*, Molec. Cell Biol. 5:281, 1985). The desmin promoter (Li *et al.*, Gene 78:243, 1989; Li *et al.*, J. Biol. Chem. 266:6562, 1991; Li *et al.*, J. Biol. Chem. 268:10403, 1993) is tissue-specific and drives expression in muscle cells. More generally, useful promoters and vectors are described, *e.g.*, in WO 94/21797 and by Hartikka *et al.* (Human Gene Therapy 7:1205, 1996).

For DNA/RNA vaccination, the polynucleotide of the invention can encode a precursor or a mature form of a polypeptide of the invention. When it encodes a precursor form, the precursor sequence can be homologous or heterologous. In the latter case, a eucaryotic leader sequence can be used, such as the leader sequence of the tissue-type plasminogen factor (tPA).

A composition of the invention can contain one or several polynucleotides of the invention. It can also contain at least one additional polynucleotide encoding another *Helicobacter* antigen, such as urease subunit A, B, or both, or a fragment, derivative, mutant, or analog thereof. A polynucleotide encoding a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12), can also be added to the composition so that the immune response is enhanced. These additional polynucleotides are placed under appropriate control for expression. Advantageously, DNA molecules of the invention

and/or additional DNA molecules to be included in the same composition are carried in the same plasmid.

Standard methods can be used in the preparation of therapeutic polynucleotides of the invention. For example, a polynucleotide can be used in a naked form, free of any delivery vehicles, such as anionic liposomes, cationic lipids, microparticles, *e.g.*, gold microparticles, precipitating agents, *e.g.*, calcium phosphate, or any other transfection-facilitating agent. In this case, the polynucleotide can be simply diluted in a physiologically acceptable solution, such as sterile saline or sterile buffered saline, with or without a carrier. When present, the carrier preferably is isotonic, hypotonic, or weakly hypertonic, and has a relatively low ionic strength, such as provided by a sucrose solution, *e.g.*, a solution containing 20% sucrose.

Alternatively, a polynucleotide can be associated with agents that assist in cellular uptake. It can be, *e.g.*, (i) complemented with a chemical agent that modifies cellular permeability, such as bupivacaine (see, *e.g.*, WO 94/16737), (ii) encapsulated into liposomes, or (iii) associated with cationic lipids or silica, gold, or tungsten microparticles.

Anionic and neutral liposomes are well-known in the art (see, *e.g.*, *Liposomes: A Practical Approach*, RPC New Ed, IRL Press, 1990, for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including polynucleotides.

Cationic lipids can also be used for gene delivery. Such lipids include, for example, LipofectinTM, which is also known as DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOTAP (1,2-bis(oleyloxy)-3-(trimethylammonio)propane), DDAB (dimethyldioctadecylammonium bromide), DOGS (dioctadecylamidoglycyl spermine), and cholesterol derivatives. A description of these cationic lipids

can be found in EP 187,702, WO 90/11092, U.S. Patent No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Patent No. 5,527,928. Cationic lipids for gene delivery are preferably used in association with a neutral lipid such as DOPE (dioleoyl phosphatidylethanolamine; WO 90/11092). Other transfection-facilitating compounds can be added to a formulation containing cationic liposomes. A number of them are described in, *e.g.*, WO 93/18759, WO 93/19768, WO 94/25608, and WO 95/2397. They include, *e.g.*, spermine derivatives useful for facilitating the transport of DNA through the nuclear membrane (see, for example, WO 93/18759) and membrane-permeabilizing compounds such as GALA, Gramicidine S, and cationic bile salts (see, for example, WO 93/19768).

Gold or tungsten microparticles can also be used for gene delivery, as described in WO 91/359, WO 93/17706, and by Tang *et al.* (Nature 356:152, 1992). In this case, the microparticle-coated polynucleotides can be injected *via* intradermal or intraepidermal routes using a needleless injection device ("gene gun"), such as those described in U.S. Patent No. 4,945,050, U.S. Patent No. 5,015,580, and WO 94/24263.

The amount of DNA to be used in a vaccine recipient depends, *e.g.*, on the strength of the promoter used in the DNA construct, the immunogenicity of the expressed gene product, the condition of the mammal intended for administration (*e.g.*, the weight, age, and general health of the mammal), the mode of administration, and the type of formulation. In general, a therapeutically or prophylactically effective dose from about 1 μ g to about 1 mg, preferably, from about 10 μ g to about 800 μ g, and, more preferably, from about 25 μ g to about 250 μ g, can be administered to human adults. The administration can be achieved in a single dose or repeated at intervals.

The route of administration can be any conventional route used in the vaccine field. As general guidance, a polynucleotide of the invention can be administered *via* a mucosal surface, *e.g.*, an ocular, intranasal, pulmonary, oral, intestinal, rectal, vaginal, or urinary tract surface, or *via* a parenteral route, *e.g.*,
5 by an intravenous, subcutaneous, intraperitoneal, intradermal, intraepidermal, or intramuscular route. The choice of administration route will depend on, *e.g.*, the formulation that is selected. A polynucleotide formulated in association with bupivacaine is advantageously administered into muscle. When a neutral or anionic liposome or a cationic lipid, such as DOTMA, is used, the
10 formulation can be advantageously injected *via* intravenous, intranasal (for example, by aerosolization), intramuscular, intradermal, and subcutaneous routes. A polynucleotide in a naked form can advantageously be administered *via* the intramuscular, intradermal, or subcutaneous routes. Although not absolutely required, such a composition can also contain an adjuvant. A
15 systemic adjuvant that does not require concomitant administration in order to exhibit an adjuvant effect is preferable.

The sequence information provided in the present application enables the design of specific nucleotide probes and primers that can be used in diagnostic methods. Accordingly, in a fifth aspect of the invention, there is
20 provided a nucleotide probe or primer having a sequence found in, or derived by degeneracy of the genetic code from, a sequence shown in any of SEQ ID NOs:1-21 (odd numbers), 65, and 67, or a complementary sequence thereof.

The term "probe" as used in the present application refers to DNA (preferably single stranded) or RNA molecules (or modifications or
25 combinations thereof) that hybridize under the stringent conditions, as defined above, to polynucleotide molecules having sequences homologous to those shown in any of SEQ ID NOs:1-21 (odd numbers), 65, and 67, or to a

complementary or anti-sense sequence of any of SEQ ID NOs:1-21 (odd numbers), 65, and 67. Generally, probes are significantly shorter than the full-length sequences shown in any of SEQ ID NOs:1-21 (odd numbers), 65, and 67. For example, they can contain from about 5 to about 100, preferably from about 10 to about 80 nucleotides. In particular, probes have sequences that are at least 75%, preferably at least 85%, more preferably 95% homologous to a portion of a sequence as shown in any of SEQ ID NOs:1-21 (odd numbers), 65, and 67, or a sequence complementary to such sequences.

Probes can contain modified bases, such as inosine, methyl-5-deoxycytidine, deoxyuridine, dimethylamino-5-deoxyuridine, or diamino-2, 6-purine. Sugar or phosphate residues can also be modified or substituted. For example, a deoxyribose residue can be replaced by a polyamide (Nielsen *et al.*, Science 254:1497, 1991) and phosphate residues can be replaced by ester groups such as diphosphate, alkyl, arylphosphonate, and phosphorothioate esters. In addition, the 2'-hydroxyl group on ribonucleotides can be modified by addition of, *e.g.*, alkyl groups.

Probes of the invention can be used in diagnostic tests, or as capture or detection probes. Such capture probes can be immobilized on solid supports, directly or indirectly, by covalent means or by passive adsorption. A detection probe can be labeled by a detectable label, for example a label selected from radioactive isotopes; enzymes, such as peroxidase and alkaline phosphatase; enzymes that are able to hydrolyze a chromogenic, fluorogenic, or luminescent substrate; compounds that are chromogenic, fluorogenic, or luminescent; nucleotide base analogs; and biotin.

Probes of the invention can be used in any conventional hybridization method, such as in dot blot methods (Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold

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Spring Harbor, New York, 1982), Southern blot methods (Southern, J. Mol. Biol. 98:503, 1975), northern blot methods (identical to Southern blot to the exception that RNA is used as a target), or a sandwich method (Dunn *et al.*, Cell 12:23, 1977). As is known in the art, the latter technique involves the use
5 of a specific capture probe and a specific detection probe that have nucleotide sequences that are at least partially different from each other.

Primers used in the invention usually contain about 10 to 40 nucleotides and are used to initiate enzymatic polymerization of DNA in an amplification process (*e.g.*, PCR), an elongation process, or a reverse
10 transcription method. In a diagnostic method involving PCR, the primers can be labeled.

Thus, the invention also encompasses (i) a reagent containing a probe of the invention for detecting and/or identifying the presence of *Helicobacter* in a biological material; (ii) a method for detecting and/or
15 identifying the presence of *Helicobacter* in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA or RNA is extracted from the material and denatured, and (c) the sample is exposed to a probe of the invention, for example, a capture probe, a detection probe, or both, under stringent hybridization conditions, so that hybridization is detected; and
20 (iii) a method for detecting and/or identifying the presence of *Helicobacter* in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA is extracted therefrom, (c) the extracted DNA is contacted with at least one, or, preferably two, primers of the invention, and amplified by the polymerase chain reaction, and (d) an amplified DNA
25 molecule is produced.

As mentioned above, polypeptides that can be produced by expression of the polynucleotides of the invention can be used as vaccine

antigens. Accordingly, a sixth aspect of the invention features a substantially purified polypeptide or polypeptide derivative having an amino acid sequence encoded by a polynucleotide of the invention.

A "substantially purified polypeptide" is defined as a polypeptide
5 that is separated from the environment in which it naturally occurs and/or a polypeptide that is free of most of the other polypeptides that are present in the environment in which it was synthesized. The polypeptides of the invention can be purified from a natural source, such as a *Helicobacter* strain, or can be produced using recombinant methods.

10 Homologous polypeptides or polypeptide derivatives encoded by polynucleotides of the invention can be screened for specific antigenicity by testing cross-reactivity with an antiserum raised against a polypeptide having an amino acid sequence as shown in any of SEQ ID NOs:2-22 (even numbers), 66, and 68. Briefly, a monospecific hyperimmune antiserum can be raised
15 against a purified reference polypeptide as such or as a fusion polypeptide, for example, an expression product of MBP, GST, or His-tag systems, or a synthetic peptide predicted to be antigenic. The homologous polypeptide or derivative that is screened for specific antigenicity can be produced as such or as a fusion polypeptide. In the latter case, and if the antiserum is also raised
20 against a fusion polypeptide, two different fusion systems are employed. Specific antigenicity can be determined using a number of methods, including Western blot (Towbin *et al.*, Proc. Natl. Acad. Sci. USA 76:4350, 1979), dot blot, and ELISA methods, as described below.

In a Western blot assay, the product to be screened, either as a
25 purified preparation or a total *E. coli* extract, is fractionated by SDS-PAGE, as described, for example, by Laemmli (Nature 227:680, 1970). After being transferred to a filter, such as a nitrocellulose membrane, the material is

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incubated with the monospecific hyperimmune antiserum, which is diluted in a range of dilutions from about 1:50 to about 1:5000, preferably from about 1:100 to about 1:500. Specific antigenicity is shown once a band corresponding to the product exhibits reactivity at any of the dilutions in the

5 range.

In an ELISA assay, the product to be screened can be used as the coating antigen. A purified preparation is preferred, but a whole cell extract can also be used. Briefly, about 100 μ l of a preparation of about 10 μ g protein/ml is distributed into wells of a 96-well ELISA plate. The plate is

10 incubated for about 2 hours at 37°C, then overnight at 4°C. The plate is washed with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBS/Tween buffer) and the wells are saturated with 250 μ l PBS containing 1% bovine serum albumin (BSA), to prevent non-specific antibody binding. After 1 hour of incubation at 37°C, the plate is washed with PBS/Tween buffer.

15 The antiserum is serially diluted in PBS/Tween buffer containing 0.5% BSA, and 100 μ l dilutions are added to each well. The plate is incubated for 90 minutes at 37°C, washed, and evaluated using standard methods. For example, a goat anti-rabbit peroxidase conjugate can be added to the wells when the specific antibodies used were raised in rabbits. Incubation is carried

20 out for about 90 minutes at 37°C and the plate is washed. The reaction is developed with the appropriate substrate and the reaction is measured by colorimetry (absorbance measured spectrophotometrically). Under these experimental conditions, a positive reaction is shown once an O.D. value of 1.0 is detected with a dilution of at least about 1:50, preferably of at least about

25 1:500.

In a dot blot assay, a purified product is preferred, although a whole cell extract can be used. Briefly, a solution of the product at a concentration of

about 100 µg/ml is serially diluted two-fold with 50 mM Tris-HCl (pH 7.5). One hundred µl of each dilution is applied to a filter, such as a 0.45 µm nitrocellulose membrane, set in a 96-well dot blot apparatus (Biorad). The buffer is removed by applying vacuum to the system. Wells are washed by

5 addition of 50 mM Tris-HCl (pH 7.5) and the membrane is air-dried. The membrane is saturated in blocking buffer (50 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 10 g/L skim milk) and incubated with an antiserum diluted from about 1:50 to about 1:5000, preferably about 1:500. The reaction is detected using standard methods. For example, a goat anti-rabbit peroxidase conjugate can be

10 added to the wells when rabbit antibodies are used. Incubation is carried out for about 90 minutes at 37°C and the blot is washed. The reaction is developed with the appropriate substrate and stopped. The reaction is then measured visually by the appearance of a colored spot, e.g., by colorimetry. Under these experimental conditions, a positive reaction is associated with detection of a

15 colored spot for reactions carried out with a dilution of at least about 1:50, preferably, of at least about 1:500. Therapeutic or prophylactic efficacy of a polypeptide or polypeptide derivative of the invention can be evaluated as is described below.

According to a seventh aspect of the invention, there is provided (i) a

20 composition of matter containing a polypeptide of the invention together with a diluent or carrier; (ii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a polypeptide of the invention; (iii) a method for inducing an immune response against *Helicobacter* in a mammal by administering to the mammal an immunogenically effective

25 amount of a polypeptide of the invention to elicit an immune response, e.g., a protective immune response to *Helicobacter*; and (iv) a method for preventing and/or treating a *Helicobacter* (e.g., *H. pylori*, *H. felis*, *H. mustelae*, or *H.*

heilmannii) infection, by administering a prophylactic or therapeutic amount of a polypeptide of the invention to an individual in need of such treatment.

Additionally, this aspect of the invention includes the use of a polypeptide of the invention in the preparation of a medicament for preventing and/or treating

5 *Helicobacter* infection.

The immunogenic compositions of the invention can be administered by any conventional route in use in the vaccine field, for example, to a mucosal (e.g., ocular, intranasal, pulmonary, oral, gastric, intestinal, rectal, vaginal, or urinary tract) surface or *via* a parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. The choice of the administration route depends upon a number of parameters, such as the adjuvant used. For example, if a mucosal adjuvant is used, the intranasal or oral route will be preferred, and if a lipid formulation or an aluminum compound is used, a parenteral route will be preferred. In the latter case, the subcutaneous or intramuscular route is most preferred. The choice of administration route can also depend upon the nature of the vaccine agent. For example, a polypeptide of the invention fused to CTB or to LTB will be best administered to a mucosal surface.

A composition of the invention can contain one or several polypeptides or derivatives of the invention. It can also contain at least one additional *Helicobacter* antigen, such as the urease apoenzyme, or a subunit, fragment, homolog, mutant, or derivative thereof.

For use in a composition of the invention, a polypeptide or polypeptide derivative can be formulated into or with liposomes, such as neutral or anionic liposomes, microspheres, ISCOMS, or virus-like particles (VLPs), to facilitate delivery and/or enhance the immune response. These compounds are readily available to those skilled in the art; for example, see

Liposomes: A Practical Approach (supra). Adjuvants other than liposomes can also be used in the invention and are well known in the art (see, for example, the list provided below).

Administration can be achieved in a single dose or repeated as
5 necessary at intervals that can be determined by one skilled in the art. For example, a priming dose can be followed by three booster doses at weekly or monthly intervals. An appropriate dose depends on various parameters, including the nature of the recipient (*e.g.*, whether the recipient is an adult or an infant), the particular vaccine antigen, the route and frequency of
10 administration, the presence/absence or type of adjuvant, and the desired effect (*e.g.*, protection and/or treatment), and can be readily determined by one skilled in the art. In general, a vaccine antigen of the invention can be administered mucosally in an amount ranging from about 10 μ g to about 500 mg, preferably from about 1 mg to about 200 mg. For a parenteral route of administration, the
15 dose usually should not exceed about 1 mg, and is, preferably, about 100 μ g.

When used as components of a vaccine, the polynucleotides and polypeptides of the invention can be used sequentially as part of a multi-step immunization process. For example, a mammal can be initially primed with a vaccine vector of the invention, such as a pox virus, *e.g.*, *via* a parenteral route,
20 and then boosted twice with a polypeptide encoded by the vaccine vector, *e.g.*, *via* the mucosal route. In another example, liposomes associated with a polypeptide or polypeptide derivative of the invention can be used for priming, with boosting being carried out mucosally using a soluble polypeptide or polypeptide derivative of the invention, in combination with a mucosal
25 adjuvant (*e.g.*, LT).

Polypeptides and polypeptide derivatives of the invention can also be used as diagnostic reagents for detecting the presence of anti-*Helicobacter*

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antibodies, *e.g.*, in blood samples. Such polypeptides can be about 5 to about 80, preferably, about 10 to about 50 amino acids in length and can be labeled or unlabeled, depending upon the diagnostic method. Diagnostic methods involving such a reagent are described below.

5 Upon expression of a polynucleotide molecule of the invention, a polypeptide or polypeptide derivative is produced and can be purified using known methods. For example, the polypeptide or polypeptide derivative can be produced as a fusion protein containing a fused tail that facilitates purification. The fusion product can be used to immunize a small mammal, *e.g.*, a mouse or
10 a rabbit, in order to raise monospecific antibodies against the polypeptide or polypeptide derivative. The eighth aspect of the invention thus provides a monospecific antibody that binds to a polypeptide or polypeptide derivative of the invention.

 By "monospecific antibody" is meant an antibody that is capable of
15 reacting with a unique, naturally-occurring *Helicobacter* polypeptide. An antibody of the invention can be polyclonal or monoclonal. Monospecific antibodies can be recombinant, *e.g.*, chimeric (*e.g.*, consisting of a variable region of murine origin and a human constant region), humanized (*e.g.*, a human immunoglobulin constant region and a variable region of animal, *e.g.*,
20 murine, origin), and/or single chain. Both polyclonal and monospecific antibodies can also be in the form of immunoglobulin fragments, *e.g.*, F(ab)'₂ or Fab fragments. The antibodies of the invention can be of any isotype, *e.g.*, IgG or IgA, and polyclonal antibodies can be of a single isotype or can contain a mixture of isotypes.

25 The antibodies of the invention, which can be raised to a polypeptide or polypeptide derivative of the invention, can be produced and identified using standard immunological assays, *e.g.*, Western blot assays, dot blot assays, or

ELISA (see, *e.g.*, Coligan *et al.*, *Current Protocols in Immunology*, John Wiley & Sons, Inc., New York, NY, 1994). The antibodies can be used in diagnostic methods to detect the presence of *Helicobacter* antigens in a sample, such as a biological sample. The antibodies can also be used in affinity chromatography methods for purifying a polypeptide or polypeptide derivative of the invention. As is discussed further below, the antibodies can also be used in prophylactic and therapeutic passive immunization methods.

Accordingly, a ninth aspect of the invention provides (i) a reagent for detecting the presence of *Helicobacter* in a biological sample that contains an antibody, polypeptide, or polypeptide derivative of the invention; and (ii) a diagnostic method for detecting the presence of *Helicobacter* in a biological sample, by contacting the biological sample with an antibody, a polypeptide, or a polypeptide derivative of the invention, so that an immune complex is formed, and detecting the complex as an indication of the presence of *Helicobacter* in the sample or the organism from which the sample was derived. The immune complex is formed between a component of the sample and the antibody, polypeptide, or polypeptide derivative, and that any unbound material can be removed prior to detecting the complex. A polypeptide reagent can be used for detecting the presence of anti-*Helicobacter* antibodies in a sample, *e.g.*, a blood sample, while an antibody of the invention can be used for screening a sample, such as a gastric extract or biopsy sample, for the presence of *Helicobacter* polypeptides.

For use in diagnostic methods, the reagent (*e.g.*, the antibody, polypeptide, or polypeptide derivative of the invention) can be in a free-state or can be immobilized on a solid support, such as, for example, on the interior surface of a tube or on the surface, or within pores, of a bead. Immobilization can be achieved using direct or indirect means. Direct means include passive

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adsorption (*i.e.*, non-covalent binding) or covalent binding between the support and the reagent. By "indirect means" is meant that an anti-reagent compound that interacts with the reagent is first attached to the solid support. For example, if a polypeptide reagent is used, an antibody that binds to it can serve
5 as an anti-reagent, provided that it binds to an epitope that is not involved in recognition of antibodies in biological samples. Indirect means can also employ a ligand-receptor system, for example, a molecule, such as a vitamin, can be grafted onto the polypeptide reagent and the corresponding receptor can be immobilized on the solid phase. This concept is illustrated by the well
10 known biotin-streptavidin system. Alternatively, indirect means can be used, *e.g.*, by adding to the reagent a peptide tail, chemically or by genetic engineering, and immobilizing the grafted or fused product by passive adsorption or covalent linkage of the peptide tail.

According to a tenth aspect of the invention, there is provided a
15 process for purifying, from a biological sample, a polypeptide or polypeptide derivative of the invention, which involves carrying out antibody-based affinity chromatography with the biological sample, wherein the antibody is a monospecific antibody of the invention.

For use in a purification process of the invention, the antibody can be
20 polyclonal or monospecific, and preferably is of the IgG type. Purified IgGs can be prepared from an antiserum using standard methods (see, *e.g.*, Coligan *et al.*, *supra*). Conventional chromatography supports, as well as standard methods for grafting antibodies, are described, for example, by Harlow *et al.* (*Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold
25 Spring Harbor, New York, 1988).

Briefly, a biological sample, such as an *H. pylori* extract, preferably in a buffer solution, is applied to a chromatography material, which is,

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preferably, equilibrated with the buffer used to dilute the biological sample, so that the polypeptide or polypeptide derivative of the invention (*i.e.*, the antigen) is allowed to adsorb onto the material. The chromatography material, such as a gel or a resin coupled to an antibody of the invention, can be in batch form or in a column. The unbound components are washed off and the antigen is eluted with an appropriate elution buffer, such as a glycine buffer, a buffer containing a chaotropic agent, *e.g.*, guanidine HCl, or a buffer having high salt concentration (*e.g.*, 3 M MgCl₂). Eluted fractions are recovered and the presence of the antigen is detected, *e.g.*, by measuring the absorbance at 280 nm.

An antibody of the invention can be screened for therapeutic efficacy as follows. According to an eleventh aspect of the invention, there is provided (i) a composition of matter containing a monospecific antibody of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a monospecific antibody of the invention; and (iii) a method for treating or preventing *Helicobacter* (*e.g.*, *H. pylori*, *H. felis*, *H. mustelae*, or *H. heilmanii*) infection, by administering a therapeutic or prophylactic amount of a monospecific antibody of the invention to an individual in need of such treatment. In addition, the eleventh aspect of the invention includes the use of a monospecific antibody of the invention in the preparation of a medicament for treating or preventing *Helicobacter* infection.

The monospecific antibody can be polyclonal or monoclonal, and is, preferably, predominantly of the IgA isotype. In passive immunization methods, the antibody is administered to a mucosal surface of a mammal, *e.g.*, the gastric mucosa, *e.g.*, orally or intragastrically, optionally, in the presence of a bicarbonate buffer. Alternatively, systemic administration, not requiring a

bicarbonate buffer, can be carried out. A monospecific antibody of the invention can be administered as a single active agent or as a mixture with at least one additional monospecific antibody specific for a different *Helicobacter* polypeptide. The amount of antibody and the particular regimen used can be readily determined by one skilled in the art. For example, daily administration of about 100 to 1,000 mg of antibody over one week, or three doses per day of about 100 to 1,000 mg of antibody over two or three days, can be effective regimens for most purposes.

Therapeutic or prophylactic efficacy can be evaluated using standard methods in the art, *e.g.*, by measuring induction of a mucosal immune response or induction of protective and/or therapeutic immunity, using, *e.g.*, the *H. felis* mouse model and the procedures described by Lee *et al.* (Eur. J. Gastroenterology & Hepatology 7:303, 1995) or Lee *et al.* (J. Infect. Dis. 172:161, 1995). Those skilled in the art will recognize that the *H. felis* strain of the model can be replaced with another *Helicobacter* strain. For example, the efficacy of polynucleotide molecules and polypeptides from *H. pylori* is, preferably, evaluated in a mouse model using an *H. pylori* strain. Protection can be determined by comparing the degree of *Helicobacter* infection in the gastric tissue assessed by, for example, urease activity, bacterial counts, or gastritis, to that of a control group. Protection is shown when infection is reduced by comparison to the control group. Such an evaluation can be made for polynucleotides, vaccine vectors, polypeptides, and polypeptide derivatives, as well as for antibodies of the invention.

For example, various doses of an antibody of the invention can be administered to the gastric mucosa of mice previously challenged with an *H. pylori* strain, as described, *e.g.*, by Lee *et al.* (*supra*). Then, after an appropriate period of time, the bacterial load of the mucosa can be estimated by

assessing urease activity, as compared to a control. Reduced urease activity indicates that the antibody is therapeutically effective.

Adjuvants that can be used in any of the vaccine compositions described above are described as follows. Adjuvants for parenteral

5 administration include, for example, aluminum compounds, such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate. The antigen can be precipitated with, or adsorbed onto, the aluminum compound using standard methods. Other adjuvants, such as RIBI (ImmunoChem, Hamilton, MT), can also be used in parenteral administration.

10 Adjuvants that can be used for mucosal administration include, for example, bacterial toxins, *e.g.*, the cholera toxin (CT), the *E. coli* heat-labile toxin (LT), the *Clostridium difficile* toxin A, the *pertussis* toxin (PT), and combinations, subunits, toxoids, or mutants thereof. For example, a purified preparation of native cholera toxin subunit B (CTB) can be used. Fragments,
15 homologs, derivatives, and fusions to any of these toxins can also be used, provided that they retain adjuvant activity. Preferably, a mutant having reduced toxicity is used. Suitable mutants are described, *e.g.*, in WO 95/17211 (Arg-7-Lys CT mutant), WO 96/6627 (Arg-192-Gly LT mutant), and WO 95/34323 (Arg-9-Lys and Glu-129-Gly PT mutant). Additional LT mutants
20 that can be used in the methods and compositions of the invention include, *e.g.*, Ser-63-Lys, Ala-69-Gly, Glu-110-Asp, and Glu-112-Asp mutants. Other adjuvants, such as the bacterial monophosphoryl lipid A (MPLA) of, *e.g.*, *E. coli*, *Salmonella minnesota*, *Salmonella typhimurium*, or *Shigella flexneri*; saponins, and polylactide glycolide (PLGA) microspheres, can also be used in
25 mucosal administration. Adjuvants useful for both mucosal and parenteral administrations, such as polyphosphazene (WO 95/2415), can also be used.

Any pharmaceutical composition of the invention, containing a polynucleotide, polypeptide, polypeptide derivative, or antibody of the invention, can be manufactured using standard methods. It can be formulated with a pharmaceutically acceptable diluent or carrier, *e.g.*, water or a saline solution, such as PBS, optionally, including a bicarbonate salt, such as sodium bicarbonate, *e.g.*, 0.1 to 0.5 M. Bicarbonate can advantageously be added to compositions intended for oral or intragastric administration. In general, a diluent or carrier can be selected on the basis of the mode and route of administration, and standard pharmaceutical practice. Suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use in pharmaceutical formulations, are described in *Remington's Pharmaceutical Sciences*, a standard reference text in this field and in the USP/NF.

The invention also includes methods in which gastroduodenal infections, such as *Helicobacter* infection, are treated by oral administration of a *Helicobacter* polypeptide of the invention and a mucosal adjuvant, in combination with an antibiotic, an antisecretory agent, a bismuth salt, an antacid, sucralfate, or a combination thereof. Examples of such compounds that can be administered with the vaccine antigen and an adjuvant are antibiotics, including, *e.g.*, macrolides, tetracyclines, β -lactams, aminoglycosides, quinolones, penicillins, and derivatives thereof (specific examples of antibiotics that can be used in the invention include, *e.g.*, amoxicillin, clarithromycin, tetracycline, metronidazole, erythromycin, cefuroxime, and erythromycin); antisecretory agents, including, *e.g.*, H_2 -receptor antagonists (*e.g.*, cimetidine, ranitidine, famotidine, nizatidine; and roxatidine), proton pump inhibitors (*e.g.*, omeprazole, lansoprazole, and pantoprazole), prostaglandin analogs (*e.g.*, misoprostil and enprostil), and anticholinergic agents (*e.g.*, pirenzepine, telenzepine, carbenoxolone, and

proglumide); and bismuth salts, including colloidal bismuth subcitrate, tripotassium dicitrate bismuthate, bismuth subsalicylate, bicitropeptide, and pepto-bismol (see, *e.g.*, Goodwin *et al.*, *Helicobacter pylori*, *Biology and Clinical Practice*, CRC Press, Boca Raton, FL, pp 366-395, 1993; Physicians' Desk Reference, 49th edn., Medical Economics Data Production Company, Montvale, New Jersey, 1995). In addition, compounds containing more than one of the above-listed components coupled together, *e.g.*, ranitidine coupled to bismuth subcitrate, can be used. The invention also includes compositions for carrying out these methods, *i.e.*, compositions containing a *Helicobacter* antigen (or antigens) of the invention, an adjuvant, and one or more of the above-listed compounds, in a pharmaceutically acceptable carrier or diluent.

Amounts of the above-listed compounds used in the methods and compositions of the invention can readily be determined by one skilled in the art. In addition, one skilled in the art can readily design treatment/immunization schedules. For example, the non-vaccine components can be administered on days 1-14, and the vaccine antigen + adjuvant can be administered on days 7, 14, 21, and 28.

Methods and pharmaceutical compositions of the invention can be used to treat or to prevent *Helicobacter* infections and, accordingly, gastroduodenal diseases associated with these infections, including acute, chronic, and atrophic gastritis, and peptic ulcer diseases, *e.g.*, gastric and duodenal ulcers.

A 76 kDa protein band containing GHPO 386, GHPO 789, and GHPO 1516 (hereinafter the "purified 76 kDa proteins"), GHPO 1360; and GHPO 750 were purified from *Helicobacter pylori* strain ATCC number 43579 (American Type Culture Collection, Rockville, Maryland) by immunoaffinity-

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based chromatography using the methods described below in Example 1, and were shown to be effective vaccine antigens as follows.

Groups of 10 mice each were orally immunized with 1, 5, or 25 µg of the purified 76 kDa proteins, purified GHPO 1360, or purified GHPO 750 in combination with 5 µg of the heat-labile enterotoxin (LT) of *E. coli*. Twenty five µg of recombinant urease, in combination with 5 µg LT, was used as a positive control, and 5 µg of LT in PBS was used as a negative control. The immunizations were carried out four times each, on days 0, 7, 14, and 21 of the experiment. On day 33, blood samples were collected from the mice and, on day 34, saliva samples were collected. On day 35, all of the mice were challenged by intragastric administration of 1×10^7 streptomycin-resistant, mouse-adapted *H. pylori*. On day 49, additional saliva samples were collected and, about two weeks after challenge, on days 52-53, the mice were sacrificed. Stomachs were removed from the mice and were analyzed for *Helicobacter* infection by measuring urease activity in the intact stomach tissue and by a quantitative culture study (Table 1).

Briefly, these studies showed that the gastric urease activities in samples from mice immunized with all three amounts of the purified 76 kDa proteins (*i.e.*, 1, 5, and 25 µg), in combination with LT, were generally lower than the gastric urease activities of samples from mice immunized with LT alone or mice that were not treated prior to challenge. Levels of gastric urease activity generally decreased with increasing amounts of the protein administered, with the gastric urease activity levels for the 25 µg doses generally approaching those of mice immunized with 25 µg of recombinant urease and LT.

The quantitative culture analyses showed that the levels of *Helicobacter* detected in the stomachs of mice immunized with the purified 76

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kDa proteins, purified GHPO 1360, or purified GHPO 750, which generally decreased with increasing dosages, were less than the levels detected in the stomachs of control mice that were immunized with LT alone or untreated before *Helicobacter* challenge (Tables 1 and 2). The percentages of mice
5 protected by immunization with the purified 76 kDa proteins, purified GHPO 1360, or purified GHPO 750 met or approached the percentages of mice protected by treatment with urease (Tables 1 and 2). These results show that the purified 76 kDa proteins, GHPO 1360, and GHPO 750 are effective vaccine antigens for use in preventing *Helicobacter* infection.

Table 1

Prophylactic Immunization with PMsv Antigens as Oral Dose Response Against <i>H. pylori</i> Challenge				
Treatment	BALB/c mice # mice infected Antrum (based on quantitative A ₅₅₀ , 0.148 O.D. cutoff)	Fisher's exact test infection status (based on quantitative A ₅₅₀ ratios, treatment group v. LT only (group 11)) p-value	CFU/ml (1/4 antrum) Mean ± SD	Wilcoxon rank sums test CFU treatment group v. LT only control (group 11) p-value
1 µg 50 kDa + LT	60% (6/10)	0.3034	30825 ± 23210	0.1736
5 µg 50 kDa + LT	40% (4/10)	0.0573	18910 ± 16341	0.0588
25 µg 50 kDa + LT	30% (3/10)	0.0198	22710 ± 32397	0.0821
1 µg 32 kDa + LT	50% (5/10)	0.1409	44225 ± 87824	0.0756
5 µg 50 kDa + LT	10% (1/10)	0.0011	11811 ± 11579	0.0191
25 µg 50 kDa + LT	0 (0/9)	0.0001	1608 ± 23917	0.0114
25 µg rUre + LT	0 (0/9)	0.0001	8208 ± 8021	0.0179
LT	90% (9/10) 90% (9/10)	- not determined	107340 ± 127949 46173 ± 42325	- 0.2568

Table 2

Prophylactic Immunization with PMsv Antigens as Oral Dose Response Against <i>H. pylori</i> Challenge				
Treatment	BALB/c mice # mice infected Antrum (based on quantitative A_{550} , 0.148 O.D. cutoff)	Fisher's exact test infection status (based on quantitative A_{550} ratios, treatment group v. LT only (group 11)) p-value	CFU/ml (1/4 antrum) Mean \pm SD	Wilcoxon rank sums test CFU treatment group v. LT only control (group 11) p-value
1 μ g 76 kDa + LT	56% (5/9)	0.1409	39922 \pm 34708	0.2203
5 μ g 76 kDa + LT	80% (4/5)	1	8802 \pm 7788	0.0864
25 μ g 76 kDa + LT	33% (3/9)	0.0198	9712 \pm 12183	0.0178
25 μ g rUre + LT	0 (0/9)	0.0001	8208 \pm 8021	0.0179
LT	90% (9/10) 90% (9/10)	- not determined	107340 \pm 127949 46173 \pm 42325	- 0.2568

- 10 The invention is further illustrated by the following examples.
- Example 1 describes purification of GHPO 1516 (76 kDa), GHPO 1360 (32 kDa), and GHPO 750 (50 kDa) from *Helicobacter* cultures. Example 2 describes identification of genes, e.g., genes encoding 76 kDa proteins, such as GHPO 386, GHPO 789, GHPO 1516, GHPO 1197, GHPO 1180, GHPO 896,
- 15 GHPO 711, GHPO 190, GHPO 185, GHPO 1417, and GHPO 1414, a 32 kDa protein (GHPO 1360), and a 50 kDa protein (GHPO 750) in the *Helicobacter* genome, as well as identification of signal sequences, and primer design for amplification of genes lacking signal sequences. Example 3 describes cloning of DNA encoding GHPO 386, GHPO 789, GHPO 1516, GHPO 896, GHPO
- 20 1360, and GHPO 750 into a vector that provides a histidine tag, and production and purification of the resulting his-tagged fusion proteins. Example 4 describes methods for cloning DNA encoding the polypeptides of the invention so that they can be produced without His-tags, Example 5 describes methods

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for purifying recombinant polypeptides of the invention, and Example 6 describes use of the GHPO 1360 polypeptide as a serodiagnostic tool for *H. pylori* infection

EXAMPLE 1: Purification and partial sequence analysis of GHPO 1516
5 **(76 kDa), GHPO 1360 (32 kDa), and GHPO 750 (50 kDa) protein from**
Helicobacter pylori

1.A. Culture and initial purification steps

Frozen seeds from *H. pylori* strain ATCC 43579 are used to seed a 75 cm² flask containing a biphasic medium (a solid phase made of Colombia
10 gelose containing 6% fresh sheep blood and a liquid phase made of triptcase soja containing 20% fetal calf serum). After 24 hours of culturing under microaerophilic conditions, the liquid phase is used for seeding several 75 cm² flasks containing biphasic medium lacking sheep blood. After 24 hours of culture, the liquid phase is used to seed a 2 L biofermentor in triptcase soja
15 liquid phase containing 10 g/L beta-cyclodextrine. At OD 1.5-1.8, this culture is diluted in a 10 L biofermentor containing the liquid medium. After 24 hours, the bacteria are spun in a centrifuge at 4,000 x g for 30 minutes at 4°C. A 10 L culture contains about 20 to 30 g (wet weight) bacteria.

The pellet obtained using the method described above is washed with
20 500 ml PBS (7.650 g NaCl, 0.724 g disodium phosphate, and 0.210 g monopotassium phosphate for one liter (pH 7.2)) for a one liter culture. The bacteria are then spun in a centrifuge again under the same conditions.

The pellet (C1) is suspended in 1% N-octyl-D-glucopyranoside (NOG; 30 ml/L; Sigma). The bacterial suspension is incubated for 1 hour at

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room temperature while stirring, spun in a centrifuge at 17,600 x g for 30 minutes at 4°C, and the pellet (C2) is recovered.

The supernatant (S2) is dialyzed against PBS overnight at 4°C while stirring. The precipitate is recovered by centrifugation at 2,600 x g for 5 30 minutes at 4°C. The supernatant (S2d) is discarded and the pellet (Cs2d) is recovered and stored at -20°C.

The pellet (C2) is resuspended in 20 mM Tris-HCl buffer (pH 7.5) and 100 µM Pefabloc (Buffer A), and is homogenized with an ultra-turrax (3821, 10 Janke and Kungel). Lysozyme and EDTA are added at 0.1 mg/ml and 1 mM, respectively.

The homogenate is sonicated three times for 2 minutes each at 4°C, and then is spun in an ultracentrifuge at 210,000 x g for 30 minutes at 4°C. The supernatant (S3), which contains the cytoplasmic and periplasmic proteins, is 15 eliminated, while the pellet is recovered, washed with buffer A, and spun in an ultracentrifuge at 210,000 x g for 30 minutes at 4°C. The supernatant (S4) is eliminated and the pellet (C4) is stored at -20°C. This pellet (C4) contains membrane proteins.

The pellet (C4) is washed in 50 mM NaCO₃ (pH 9.5) and 100 µM 20 Pefabloc (buffer B). The suspension is spun in an ultracentrifuge at 210,000 x g for 30 minutes at 4°C. The supernatant (S5) is eliminated, and the pellet (C5) is then washed and spun in an ultracentrifuge as is described above. The supernatant (S6) is eliminated and the pellet (C6) is stored at -20°C.

1.B. Purification of the proteins of membrane fraction C4 by preparative SDS-PAGE.

SDS-PAGE is carried out according to the method of Laemmli (*supra*), using a biphasic gel consisting of a 5% polyacrylamide concentrating gel and a 10% polyacrylamide separating gel. The membrane fraction C4 is resuspended in buffer A, diluted in an equal volume of 2x sample buffer, and heated for 5 minutes at 95°C. About 19 mg of protein is applied to the gel (16 x 12 cm; 5 mm thick). Pre-migration is carried out for 2 hours at 50 V, and is followed by migration overnight at 65 V. After Coomassie blue staining, five major bands are revealed that have apparent molecular weights of 87, 76, 54, 50, and 32 kDa. Bands at 50 and 32 kDa appear to be slightly contaminated with bands at 47 and 35 kDa, respectively.

A band corresponding to the purified 76 kDa proteins, 32 kDa protein (GHPO 1360), or 50 kDa protein (GHPO 750) is cut out from the gel and is pounded with an ultra-turrax in 10-20 ml extraction buffer (25 mM Tris-HCl (pH 8.8), 8 M urea, 10% SDS, 100 μ M phenyl methyl sulfonyl fluoride (PMSF), and 10 μ M Pefabloc (buffer C)).

Each homogenate is filtered through a Millipore AP20 filter under 7 bars at room temperature, washed with 5-10 ml buffer C, and then filtered again. Each filtrate is precipitated with three volumes of a 50/50 mixture of 75% methanol and 75% isopropanol, and then is spun in a centrifuge at 240,000 x g for 16 hours at 10°C.

Each pellet is resuspended in 2 ml of 10 mM NaPO₄ (pH 7.0) containing 1 M NaCl, 0.1% Sarkosyl, 100 μ M PMSF, and 6 M urea (buffer D). The solubilized sample is dialyzed, in order, against 100 ml buffer D containing 4 M urea, 100 ml buffer D containing 2 M urea and 0.5% Sarkosyl, and twice against 100 ml buffer D that does not contain urea or Sarkosyl. The dialyses

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are carried out for 1 hour each while stirring at room temperature. The last dialysate is incubated for 30 minutes in an ice bath, and then is spun in a centrifuge at low speed for 10 minutes at 4°C. The supernatant is recovered, filtered through a Millipore filter (0.45 µm), and stored at -20°C.

5 **1.C. Purification of the 76 kDa, 32 kDa, or 50 kDa protein by immunoaffinity-based chromatography**

1.C.1. Antiserum preparation

Specific polyclonal serum against the purified 76 kDa proteins, the 32 kDa protein (GHPO 1360), or the 50 kDa protein (GHPO 750), which are
10 purified by preparative SDS-PAGE, is prepared by hyperimmunizing rabbits as follows. On day 0, a preparation containing 50 µg of the protein mixed with complete Freund's adjuvant is administered subcutaneously to the rabbits at multiple sites. The rabbits are boosted at days 21 and 42 with 25 µg of the protein in incomplete Freund's adjuvant, and are sacrificed at day 60.

15 Complement is removed from the serum by heating for 30 minutes at 56°C. The hyperimmune serum is then sterilized by filtration through a Millipore membrane (0.22 µm).

1.C.2. IgG purification

The hyperimmune serum prepared as described above is applied to a
20 Protein A Sepharose Fast Flow column (Pharmacia) that is equilibrated with 100 mM Tris-HCl (pH 8.0). The column is washed with 10 column volumes of 100 mM Tris-HCl (pH 8.0), and then with 10 column volumes of 10 mM Tris-HCl (pH 8.0). IgGs are eluted in 0.1 M glycine buffer (pH 3.0), and are
collected as 5 ml fractions, to each of which 0.25 ml of Tris-HCl (pH 8.0) is
25 added. The optical density of each fraction is measured at 280 nm, the IgG-containing fractions are pooled together and, if necessary, frozen at -70°C.

1.C.3. Preparation of the column

An appropriate amount of CNBr-activated Sepharose 4B gel (Pharmacia; reference: 17-0430-01) is suspended in 1 mM NaCl buffer (1 g dry gel provides for 3.5 ml hydrated gel; 5 to 10 mg IgGs can be retained per ml of hydrated gel). The gel is then washed using a buchner by adding small quantities of 1 mM HCl. The total volume of 1 mM HCl that is used amounts to 200 ml/g of gel.

Purified IgGs are dialyzed for 4 hours at room temperature against 50 volumes of 500 mM sodium phosphate buffer (pH 7.5). The IgGs are then diluted to 3 mg/ml with the same buffer. IgGs are incubated with the gel overnight at $5 \pm 3^\circ\text{C}$ while stirring. The gel is packed in a chromatography column and is washed with 2 column volumes of 500 mM phosphate buffer (pH 7.5). The gel is then transferred to a tube and is incubated with 100 mM ethanolamine (pH 7.5), and then it is washed with 2 column volumes of PBS. The gel can be stored in PBS/merthiolate, 1/10,000.

1.C.4. Adsorption and elution

The 76 kDa protein is adsorbed and eluted as follows. The membrane fraction Cs2d is suspended in 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, and then is filtered through a 0.45 μm membrane. The supernatant is applied to the column, which is equilibrated with 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, at a flow rate of about 10 ml/hour. The column is washed with 20 column volumes of 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, and then with 2 to 6 volumes 10 mM phosphate buffer (pH 6.8).

The antigen is eluted with 100 mM glycine buffer (pH 2.5). The eluate is collected in 3 ml fractions, to each of which is added 150 μl 1 M phosphate buffer (pH 8.0). The optical density of each fraction is measured at

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280 nm, fractions containing the 76 kDa protein are pooled, and stored at -70°C.

Analysis by 10% SDS-PAGE reveals a single band at 76 kDa. N-terminal sequence was carried out on this purified 76 kDa preparation, and the sequence obtained is as follows: EDDGFYTSVGYQIGEEAAQMV (SEQ ID NO:58).

The 32 kDa protein (GHPO 1360) or the 50 kDa protein (GHPO 750) is purified by immunoaffinity-based chromatography as follows. In order to separate the 32 or 50 kDa protein from the contaminating proteins (the 47 and 35 kDa proteins, respectively), membrane fraction C4 is solubilized in 50 mM NaCO₃ (pH 9.5) for 30 minutes at room temperature under stirring and the preparation is centrifuged for 30 minutes at 200,000 x g at 4°C. The 47 and 35 kDa proteins are insoluble in the NaCO₃ buffer and are eliminated in the pellet.

The supernatant is dialyzed against 50 mM Tris-HCL (pH 8.0), 2 mM EDTA, and then is filtered through a 0.45 µm membrane. The filtered supernatant is applied to the column, which is equilibrated with 50 mM Tris-HCL (pH 8.0), 2 mM EDTA, at a flow rate of about 10 ml/hour. The column is washed with 20 column volumes of 50 mM Tris-HCL (pH 8.0), 2 mM EDTA, and then with 2 to 6 volumes of 10 mM phosphate buffer (pH 6.8).

The antigen is eluted with 100 mM glycine buffer (pH 2.5). The eluate is collected in 3 ml fractions, to each of which is added 150 µl 1 M phosphate buffer (pH 8.0). The optical density of each fraction is measured at 280 nm, and fractions containing the 50 or 32 kDa protein are pooled and stored at -70°C.

Analysis of the purified protein by 10% SDS-PAGE reveals single bands at 50 and 32 kDa. N-terminal sequencing is carried out with the purified 50 kDa protein preparation. The sequence found is as follows: MKEKFNRTKPHVNIGTIGHVDH (SEQ ID NO:73). Similarly, N-terminal

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and internal sequencing is carried out with the purified 32 kDa preparation. The sequences found are as follows: AHNANNATHNTKK (SEQ ID NO:74) and KPAHNA (SEQ ID NO:75) (N-terminal), and IDKQPKAKK (SEQ ID NO:76) and FWAKKQAE (SEQ ID NO:77) (internal).

5 **1.D. Purification of the 76 kDa protein from membrane fraction Cs2d and purification of the 32 kDa and 50 kDa proteins from membrane fraction C4**

10 The 76 kDa protein can also be purified as follows. A 40 ml Q-Sepharose column (diameter: 2.5 cm; height: 8 cm) is prepared according to the manufacturer's instructions (Pharmacia). The column is washed and equilibrated with buffer B, containing 50 mM NaCO₃ (pH 9.5), 100 µM Pefabloc, and 0.1% Zwittergent 3-14. The chromatography is monitored by measuring absorbance at 280 nm at the column exit.

15 One hundred and forty mg of protein from the membrane fraction Cs2d resuspended in buffer B are applied to the column. The column is washed with 0.1 M NaCl in buffer B, and then a 0.1-0.5 M NaCl gradient is applied to the column. The fraction eluted between 0.35 and 0.45 M NaCl is further purified on a 10 ml S-Sepharose column (diameter: 1.5 cm; height: 5 cm; up to 10 mg protein/ml of gel), which is prepared according to the
20 manufacturer's instructions (Pharmacia). The fraction obtained is dialyzed against 50 mM acetate (pH 5.0) containing 100 µM Pefabloc and 0.1% Zwittergent 3-14, and then is applied to the column, which is equilibrated with the acetate buffer.

25 The column is washed with the acetate buffer until the absorbance at 280 nm is stabilized (about 3 column volumes are required). Proteins are

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eluted with a 0-0.5 M NaCl gradient in acetate buffer. The fraction eluted at 0.15 M NaCl is enriched with the 76 kDa protein.

The 32 kDa protein (GHPO 1360) can also be purified as follows.

Membrane fraction C4 is solubilized in 50 mM NaCO₃ buffer (pH 9.5) at room
5 temperature for 30 minutes under stirring. The suspension is then centrifuged
at 200,000 x g for 30 minutes at 4°C. This allows the 32 and 35 kDa proteins
to be separated, since the 35 kDa protein is insoluble in the NaCO₃ buffer. The
supernatant is dialyzed against 50 mM NaPO₄ buffer (pH 7.0), and then is
applied to an SP-Sepharose column, which is equilibrated with the NaPO₄
10 buffer. The column is washed with the NaPO₄ buffer, and then an 0-0.5 M
NaCl gradient is applied to the column. The fraction eluted between 0.26 and
0.31 M contains the 32 kDa protein.

The 50 kDa protein can also be purified as follows. Membrane fraction
C4 is solubilized in 50 mM NaCO₃ buffer (pH 9.5) at room temperature for
15 30 minutes while stirring. The suspension is then centrifuged at 200,000 x g
for 30 minutes at 4°C. This allows the 50 and 47 kDa proteins to be separated,
since the 47 kDa protein is insoluble in the NaCO₃ buffer. The supernatant is
dialyzed against 50 mM NaPO₄ buffer (pH 7.0).

A 40 ml Q-Sepharose column (diameter: 2.5 cm; height: 8 cm) is
20 prepared according to the manufacturer's instructions (Pharmacia), washed, and
equilibrated with buffer B (pH 9.5) (50 mM NaCO₃, 100 µM Pefabloc, and
0.1% Zwittergent 3-14).

The chromatography is monitored by UV detection at 280 nm at the
column exit. One hundred and forty mg of protein solubilized as is described
25 above are applied to the column, which is then washed with buffer B until the
absorbance at 280 nm is stabilized. The proteins are eluted with a 0.1-
0.5 M NaCl gradient in buffer B (10 fold V_T), which is followed by washing in

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buffer B containing 0.5, and then 1, M NaCl (2 fold V_T). The fractions are recovered, analyzed by SDS-PAGE, and pooled according to their electrophoretic profiles.

Fraction 9, which corresponds to the beginning of the washing at 1 M NaCl and contains acidic proteins, is further purified as follows. A 10 ml DEAE Sepharose column (diameter: 1.5 cm, height: 5 cm) is prepared according to the manufacturer's instructions (Pharmacia) (up to 10 mg protein/ml of gel). The column is washed and equilibrated with buffer B. Chromatography is monitored as is described above.

Fraction 9 is dialyzed against buffer B and contains about 10 mg protein. Fraction 9 is applied to the DEAE-Sepharose column. The column is washed with buffer B until the absorbance at 280 nm is stabilized. The proteins are eluted with a 0-0.5 M NaCl gradient in buffer B (10 fold V_T), followed by washing in buffer B, containing 1 M NaCl (2 fold V_T). Fractions are recovered and analyzed by SDS-PAGE. The 50 kDa protein is found in the fractions eluted at 0.3-0.4 M NaCl.

EXAMPLE 2: Identification of genes in the *H. pylori* genome, such as genes encoding the 76 kDa proteins, the 32 kDa protein (GHPO 1360), and the 50 kDa protein (GHPO 750) identification of signal sequences, and primer design for amplification of genes lacking signal sequences

2.A. Creating *H. pylori* genomic databases

The *H. pylori* genome was provided as a text file containing a single contiguous string of nucleotides that had been determined to be 1.76 Megabases in length. The complete genome was split into 17 separate files using the program SPLIT (Creativity in Action), giving rise to 16 contigs, each

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containing 100,000 nucleotides, and a 17th contig containing the remaining 76,000 nucleotides. A header was added to each of the 17 files using the format: >hpg0.txt (representing contig 1), .hpg1.txt (representing contig 2), etc. The resulting 17 files, named hpg0 through hpg16, were then copied together to form one file that represented the plus strand of the complete *H. pylori* genome. The constructed database was given the designation "H." A negative strand database of the *H. pylori* genome was created similarly by first creating a reverse complement of the positive strand using the program SeqPup (D.G. Gilbert, Indiana University Biology Department) and then performing the same procedure as described above for the plus strand. This database was given the designation "N."

The regions predicted to encode open reading frames (ORFs) were defined for the complete *H. pylori* genome using the program GENEMARK™ (Borodovsky *et al.*, Comp. Chem. 17:123, 1993). A database was created from a text file containing an annotated version of all ORFs predicted to be encoded by the *H. pylori* genome for both the plus and minus strands, and was given the designation "O." Each ORF was assigned a number indicating its location on the genome and its position relative to other genes. No manipulation of the text file was required.

20 **2.B. Searching the *H. pylori* databases**

The databases constructed as is described above were searched using the program FASTA (Pearson *et al.*, Proc. Natl. Acad. Sci. USA 85:2444-2448, 1988). FASTA was used for searching either a DNA sequence against either of the gene databases ("H" and/or "N"), or a peptide sequence against the ORF library ("O"). TFASTX was used to search a peptide sequence against all possible reading frames of a DNA database ("H" and/or "N" libraries).

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Potential frameshifts also being resolved, FASTX was used for searching the translated reading frames of a DNA sequence against either a DNA database, or a peptide sequence against the protein database.

2.C. Isolation of DNA sequences from the *H. pylori* genome

- 5 The FASTA searches against the constructed DNA databases identified exact nucleotide coordinates on one or more of the isolated contigs, and therefore the location of the target DNA. Once the exact location of the target sequence was known, the contig identified to carry the gene was exported into the software package MapDraw (DNASTar, Inc.) and the gene was isolated.
- 10 Gene sequences with flanking DNA was then excised and copied into the EditSeq. Software package (DNASTar, Inc.) for further analysis.

2.D. Identification of signal sequences

- The deduced protein encoded by a target gene sequence is analyzed using the PROTEAN software package (DNASTar, Inc.). This analysis predicts
- 15 those areas of the protein that are hydrophobic by using the Kyte-Doolittle algorithm, and identifies any potential polar residues preceding the hydrophobic core region, which is typical for many signal sequences. For confirmation, the target protein is then searched against a PROSITE database (DNASTar, Inc.) consisting of motifs and signatures. Characteristic of many
- 20 signal sequences and hydrophobic regions in general, is the identification of predicted prokaryotic lipid attachment sites. Where confirmation between the two approaches is apparent at the N-terminus of any protein, putative cleavage sites are sought. Specifically, this includes the presence of either an Alanine (A), Serine (S), or Glycine (G) residue immediately after the core hydrophobic

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region. In the case of lipoproteins, a Cysteine (C) residue would be identified as the +1 residue, post-cleavage.

2.E. Rational design of PCR primers based on the identification of signal sequences

5 In order to clone gene sequences as N-terminus translational fusions for the generation of recombinant proteins with N-terminal Histidine tags, the gene sequence that specifies the signal sequence is omitted. The 5'-end of the gene-specific portion of the N-terminal primer is designed to start at the first codon beyond the cleavage site. In the case of lipoproteins, the 5'-end of the N-
10 terminal primer begins at the second codon, immediately after the modifiable residue at position +1 post-cleavage. The omission of the signal sequence from the recombinant allows for one-step purification, and potential problems associated with insertion of signal sequences in the membrane of the host strain carrying the hybrid construct are avoided.

15 **EXAMPLE 3: Preparation of isolated DNA encoding GHPO 386, GHPO 789, GHPO 1516, GHPO 896, GHPO 1360, and GHPO 750, and production of these proteins as a histidine-tagged fusion proteins**

3.A. Preparation of genomic DNA from *Helicobacter pylori*

20 *Helicobacter pylori* strain ORV2001, stored in LB medium containing 50% glycerol at -70°C, is grown on Colombia agar containing 7% sheep blood for 48 hours under microaerophilic conditions (8-10% CO₂, 5-7% O₂, and 85-87% N₂). Cells are harvested, washed with PBS (pH 7.2), and DNA is then extracted from the cells using the Rapid Prep Genomic DNA Isolation kit (Pharmacia Biotech).

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3.B. PCR amplification

DNA encoding GHPO 386, GHPO 789, GHPO 1516, GHPO 896, GHPO 1360, and GHPO odd numbers), 65, and 67 is amplified from genomic DNA, as can be prepared as is described above, by the Polymerase Chain

5 Reaction (PCR) using the following primers:

GHPO 386:

N-terminal primer:

5'-CTGAATTCGATTTCAAGGAGAAAACATGAAA-3' (SEQ ID NO:59);

and

10 C-terminal primer:

5'-CCGCTCGAGTTAGTAAGCGAACACATAATT-3' (SEQ ID NO:60).

GHPO 789:

N-terminal primer:

5'-CGCGGATCCGAATCCAATTTAATCCAAAAAGG-3' (SEQ ID NO:61);

15 and

C-terminal primer:

5'-CCGCTCGAGTTAGTAAGCGAACACATAGTTCAA-3' (SEQ ID NO:62).

GHPO 1516:

N-terminal primer:

20 5'-CGCGGATCCGAATCCAATTTAATCCAAAAAGG-3' (SEQ ID NO:56);

and

C-terminal primer:

5'-CCGCTCGAGTTAAGTAAGCGAACACATATTCAA-3' (SEQ ID NO:57).

GHPO 896:

25 N-terminal primer:

5'-CGCGGATCCGAAGTTTCTTTGTATCAAAG-3' (SEQ ID NO:63); and

C-terminal primer:

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5'-CCGCTCGAGTTAGTAAGCAAACACATAATTGTG-3' (SEQ ID NO:64).

GHPO 1360:

N-terminal primer:

5'-CGCGGATCCGAATGAAAAAAAAATATCTTAAAT-3' (SEQ ID NO:69);

5 and

C-terminal primer:

5'-CCGCTCGAGTTACTTGTTGATAACAATTTT-3' (SEQ ID NO:70).

GHPO 750:

N-terminal primer:

10 5'-CGCGGATCCGAATGGCAAAGAAAAGTTTAAC-3' (SEQ ID NO:71);

and

C-terminal primer:

5'-CCGCTCGAGTTATTCAATAATATTGCTCAC-3' (SEQ ID NO:72).

GHPO 711:

15 N-terminal primer:

5'-GGGAATTCAAAAAACGAAAAAACG-3' (SEQ ID NO:83); and

C-terminal primer:

5'-CCCCTCGAGTTAATAGGCAAACAC-3' (SEQ ID NO:84).

20 The N-terminal and C-terminal primers for each clone both include a 5' clamp and a restriction enzyme recognition sequence for cloning purposes (*Bam*HI (GGATCC) and *Xho*I (CTCGAG) recognition sequences).

Amplification of gene-specific DNA is carried out using a heat-stable DNA Polymerase (*e.g.*, Thermalase DNA Polymerase (Amresco)) according to the manufacturer's instructions. The reaction mixture, which is brought to a

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final volume of 100 μ l with distilled water, is as follows:

	dNTPs mix	200 μ M
	10x ThermoPol buffer	10 μ l
5	primers	300 nM each
	DNA template	50 ng
	DNA polymerase	2 units

Appropriate amplification reaction conditions can readily be determined by one skilled in the art. In the present case, the following conditions were used. For GHPO 386 and GHPO 789, in a reaction containing Taq DNA polymerase (Appligene), a denaturing step was carried out at 95°C for 30 seconds, followed by an annealing step at 50°C for one minute, and an extension step at 72°C for 2 minutes and 30 seconds. Twenty five cycles were carried out. For GHPO 896, in a reaction containing Taq DNA polymerase, a denaturing step was carried out at 97°C for 30 seconds, followed by an annealing step at 50°C for one minute, and an extension step at 72°C for 2 minutes and 30 seconds. Twenty five cycles were carried out. The same reaction conditions were used for GHPO 1516 as GHPO 896, except that Vent DNA polymerase was used for clone GHPO 1516, instead of Taq DNA polymerase, and the annealing temperature was 55°C. For GHPO 1360 and GHPO 750, Thermalase DNA polymerase was used. A denaturing step was carried out at 95°C for 30 seconds, followed by an annealing step at 55°C for one minute, and an extension step at 72°C for 2 minutes. Thirty cycles were carried out. For GHPO 711, Vent DNA polymerase was used. A denaturing step was carried out at 94°C for 30 seconds, followed by an annealing step at 50°C for 30 seconds, and an extension step at 72°C for 1 minute. Twenty five cycles were carried out.

3.C. Transformation and selection of transformants

A single PCR product is thus amplified and is then digested at 37°C for 2 hours with *Bam*HI and *Xho*I concurrently in a 20 µl reaction volume. The digested product is ligated to similarly cleaved pET28a (Novagen) that is dephosphorylated prior to the ligation by treatment with Calf Intestinal Alkaline Phosphatase (CIP). The gene fusion constructed in this manner allows one-step affinity purification of the resulting fusion protein because of the presence of histidine residues at the N-terminus of the fusion protein, which are encoded by the vector.

The ligation reaction (20 µl) is carried out at 14°C overnight and then is used to transform 100 µl fresh *E. coli* XL1-blue competent cells (Novagen). The cells are incubated on ice for 2 hours, then heat-shocked at 42°C for 30 seconds, and returned to ice for 90 seconds. The samples are then added to 1 ml LB broth in the absence of selection and grown at 37°C for 2 hours. The cells are then plated out on LB agar containing kanamycin (50 µg/ml) at a 10x and neat dilution and incubated overnight at 37°C. The following day, 50 colonies are picked onto secondary plates and incubated at 37°C overnight.

Five colonies are picked into 3 ml LB broth supplemented with kanamycin (100 µg/ml) and are grown overnight at 37°C. Plasmid DNA is extracted using the Quiagen mini-prep. method and is quantitated by agarose gel electrophoresis.

PCR is performed with the gene-specific primers under the conditions stated above and transformant DNA is confirmed to contain the desired insert. If PCR-positive, one of the five plasmid DNA samples (500 ng) extracted from the *E. coli* XL1-blue cells is used to transform competent BL21 (λDE3) *E. coli* competent cells (Novagen; as described previously). Transformants (10) are

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picked onto selective kanamycin (50 µg/ml) containing LB agar plates and stored as a research stock in LB containing 50% glycerol.

3.D. Purification of recombinant proteins

One ml of frozen glycerol stock prepared as described in 3.C. is used to
5 inoculate 50 ml of LB medium containing 25 µg/ml of kanamycin in a 250 ml Erlenmeyer flask. The flask is incubated at 37°C for 2 hours or until the absorbance at 600 nm (OD_{600}) reaches 0.4-1.0. The culture is stopped from growing by placing the flask at 4°C overnight. The following day, 10 ml of the overnight culture are used to inoculate 240 ml LB medium containing
10 kanamycin (25 µg/ml), with the initial OD_{600} about 0.02-0.04. Four flasks are inoculated for each ORF.

The cells are grown to an OD_{600} of 1.0 (about 2 hours at 37°C), a 1 ml sample is harvested by centrifugation, and the sample is analyzed by SDS-PAGE to detect any leaky expression. The remaining culture is induced with 1
15 mM IPTG and the induced cultures are grown for an additional 2 hours at 37°C.

The final OD_{600} is taken and the cells are harvested by centrifugation at 5,000 x g for 15 minutes at 4°C. The supernatant is discarded and the pellets are resuspended in 50 mM Tris-HCl (pH 8.0), 2 mM EDTA. Two hundred and
20 fifty ml of buffer are used for a 1 L culture and the cells are recovered by centrifugation at 12,000 x g for 20 minutes. The supernatant is discarded and the pellets are stored at -45°C.

3. E. Protein purification

Pellets obtained from 3.D. are thawed and resuspended in 95 ml of 50
25 mM Tris-HCl (pH 8.0). Pefabloc and lysozyme are added to final

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concentrations of 100 μ M and 100 μ g/ml, respectively. The mixture is homogenized with magnetic stirring at 5°C for 30 minutes. Benzonase (Merck) is added at a 1 U/ml final concentration, in the presence of 10 mM $MgCl_2$, to ensure total digestion of the DNA. The suspension is sonicated (Branson
5 Sonifier 450) for 3 cycles of 2 minutes each at maximum output. The homogenate is spun in a centrifuge at 19,000 x g for 15 minutes and both the supernatant and the pellet are analyzed by SDS-PAGE to detect the cellular location of the target protein in the soluble or insoluble fractions, as is described further below.

10 3.E.1. Soluble fraction

If the target protein is produced in a soluble form (*i.e.*, in the supernatant obtained in 3.E.) NaCl and imidazole are added to the supernatant to final concentrations of 50 mM Tris-HCl (pH 8.0), 0.5 M NaCl, and 10 mM imidazole (buffer A). The mixture is filtered through a 0.45 μ m membrane and
15 loaded onto an IMAC column (Pharmacia HiTrap chelating Sepharose; 1 ml) that has been charged with nickel ions according to the manufacturer's recommendations. After loading, the column is washed with 50 column volumes of buffer A and the recombinant target protein is eluted with 5 ml of buffer B (50 mM Tris-HCl (pH 8.0), 0.5 M NaCl, 500 mM imidazole).

20 The elution profile is monitored by measuring the absorbance of the fractions at 280 nm. Fractions corresponding to the protein peak are pooled, dialyzed against PBS containing 0.5 M arginine, filtered through a 0.22 μ m membrane, and stored at -45°C.

3.E.2. Insoluble fraction

25 If the target protein is expressed in the insoluble fraction (pellets obtained from 3.E.), purification is conducted under denaturing conditions. NaCl, imidazole, and urea are added to the resuspended pellet to final

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concentrations of 50 mM Tris-HCl (pH 8.0), 0.5 M NaCl, 10 mM imidazole, and 6 M urea (buffer C). After complete solubilization, the mixture is filtered through a 0.45 μ m membrane and loaded onto an IMAC column.

The purification procedures on the IMAC column are the same as described in 3.E.1., except that 6 M urea is included in all buffers used and 10 column volumes of buffer C are used to wash the column after protein loading, instead of 50 column volumes.

The protein fractions eluted from the IMAC column with buffer D (buffer C containing 500 mM imidazole) are pooled. Arginine is added to the solution to final concentration of 0.5 M and the mixture is dialyzed against PBS containing 0.5 M arginine and various concentrations of urea (4 M, 3 M, 2 M, 1 M, and 0.5 M) to progressively decrease the concentration of urea. The final dialysate is filtered through a 0.22 μ m membrane and stored at -45°C.

Alternatively, when the above purification process is not as efficient as it should be, two other processes may be used as follows. A first alternative involves the use of a mild denaturant, N-octyl glucoside (NOG). Briefly, a pellet obtained in 3.E. is homogenized in 5 mM imidazole, 500 mM sodium chloride, 20 mM Tris-HCl (pH 7.9) by microfluidization at a pressure of 15,000 psi and is clarified by centrifugation at 4,000-5,000 x g. The pellet is recovered, resuspended in 50 mM NaPO₄ (pH 7.5) containing 1-2% weight /volume NOG, and homogenized. The NOG-soluble impurities are removed by centrifugation. The pellet is extracted once more by repeating the preceding extraction step. The pellet is dissolved in 8 M urea, 50 mM Tris (pH 8.0). The urea-solubilized protein is diluted with an equal volume of 2 M arginine, 50 mM Tris (pH 8.0), and is dialyzed against 1 M arginine for 24-48 hours to remove the urea. The final dialysate is filtered through a 0.22 μ m membrane and stored at -45°C.

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A second alternative involves the use of a strong denaturant, such as guanidine hydrochloride. Briefly, a pellet obtained in 3.E. is homogenized in 5 mM imidazole, 500 mM sodium chloride, 20 mM Tris-HCl (pH 7.9) by microfluidization at a pressure of 15,000 psi and clarified by centrifugation at 4,000-5,000 x g. The pellet is recovered, resuspended in 6 M guanidine hydrochloride, and passed through an IMAC column charged with Ni⁺⁺. The bound antigen is eluted with 8 M urea (pH 8.5). Beta-mercaptoethanol is added to the eluted protein to a final concentration of 1 mM, then the eluted protein is passed through a Sephadex G-25 column equilibrated in 0.1 M acetic acid.

Protein eluted from the column is slowly added to 4 volumes of 50 mM phosphate buffer (pH 7.0). The protein remains in solution.

3.F. Evaluation of the protective activity of the purified protein

A protection test is described above that was carried out for testing the protective activity of the purified, native proteins. This test can also be used for testing the protective efficacy of recombinant proteins. Alternatively, the following test can be used.

Groups of 10 OF1 mice (IFFA Credo) are immunized rectally with 25 µg of the purified recombinant protein, admixed with 1 µg of cholera toxin (Berna) in physiological buffer. Mice are immunized on days 0, 7, 14, and 21. Fourteen days after the last immunization, the mice are challenged with *H. pylori* strain ORV2001 grown in liquid media (the cells are grown on agar plates, as described in 1.A., and, after harvest, the cells are resuspended in Brucella broth; the flasks are then incubated overnight at 37°C). Fourteen days after challenge, the mice are sacrificed and their stomachs are removed. The amount of *H. pylori* is determined by measuring the urease activity in the stomach and by culture.

3.G. Production of monospecific polyclonal antibodies

3.G.1. Hyperimmune rabbit antiserum

New Zealand rabbits are injected both subcutaneously and intramuscularly with 100 µg of a purified fusion polypeptide, as obtained in
5 3.E.1. or 3.E.2., in the presence of Freund's complete adjuvant and in a total volume of approximately 2 ml. Twenty one and 42 days after the initial injection, booster doses, which are identical to priming doses, except that Freund's incomplete adjuvant is used, are administered in the same way. Fifteen days after the last injection, animal serum is recovered,
10 decomplemented, and filtered through a 0.45 µm membrane.

3.G.2. Mouse hyperimmune ascites fluid

Ten mice are injected subcutaneously with 10-50 µg of a purified fusion polypeptide, as obtained in 3.E.1. or 3.E.2., in the presence of Freund's complete adjuvant and in a volume of approximately 200 µl. Seven and 14
15 days after the initial injection, booster doses, which are identical to the priming doses, except that Freund's incomplete adjuvant is used, are administered in the same way. Twenty one and 28 days after the initial infection, mice receive 50 µg of the antigen alone intraperitoneally. On day 21, mice are also injected intraperitoneally with sarcoma 180/TG cells CM26684 (Lennette *et al.*,
20 *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*, 5th Ed., Washington DC, American Public Health Association, 1979). Ascites fluid is collected 10-13 days after the last injection.

EXAMPLE 4: Methods for producing transcriptional fusions lacking His-tags

25 Methods for amplification and cloning of DNA encoding the polypeptides of the invention as transcriptional fusions lacking His-tags are

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described as follows. Two PCR primers for each clone are designed based upon the sequences of the polynucleotides that encode them (SEQ ID NOs:1-21 (odd numbers), 65, and 67). These primers can be used to amplify DNA encoding the polypeptides of the invention from any *Helicobacter pylori* strain, including, for example, ORV2001 and the *H. pylori* strain deposited with the American Type Culture Collection (ATCC, Rockville, Maryland) as ATCC number 43579, as well as from other *Helicobacter* species.

The N-terminal primers are designed to include the ribosome binding site of the target gene, the ATG start site, the signal sequence (if any), and the cleavage site. The N-terminal primers can include a 5' clamp and restriction endonuclease recognition site, such as that for *Bam*HI (GGATCC), which facilitates subsequent cloning. Similarly, the C-terminal primers can include a restriction endonuclease recognition site, such as that for *Xho*I (CTCGAG), which can be used in subsequent cloning, and a TAA stop codon. Specific primers that can be used are listed above.

Amplification of a genes encoding the polypeptides of the invention can be carried out using Vent DNA polymerase (New England Biolabs) or Taq DNA polymerase (Appligene) under the conditions described above in Example 3. Alternatively, Thermalase DNA polymerase or Pwo DNA polymerase (Boehringer Mannheim) can be used, according to instructions provided by the manufacturers.

A single PCR product for each clone is amplified and can be cloned into *Bam*HI-*Xho*I cleaved pET24, resulting in construction of transcriptional fusions that permit expression of the proteins without His-tags. The expressed products can be purified as denatured proteins that are refolded by dialysis into 1 M arginine.

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Cloning into pET 24 allows transcription of genes from the T7 promoter, which is supplied by the vector, but relies upon binding of the RNA-specific DNA polymerase to the intrinsic ribosome binding site of the genes, and thereby expression of the complete ORF. The amplification, digestion, and
5 cloning protocols are as described above for constructing translational fusions.

EXAMPLE 5: Purification of the polypeptides of the invention by immunoaffinity

5.A. Purification of specific IgGs

An immune serum, as prepared as is described in section 3.G., is applied
10 to a protein A Sepharose Fast Flow column (Pharmacia) equilibrated in 100 mM Tris-HCl (pH 8.0). The resin is washed by applying 10 column volumes of 100 mM Tris-HCl and 10 volumes of 10 mM Tris-HCl (pH 8.0) to the column. IgG antibodies are eluted with 0.1 M glycine buffer (pH 3.0) and are collected in 5 ml fractions to each of which is added 0.25 ml 1 M Tris-HCl
15 (pH 8.0). The optical density of the eluate is measured at 280 nm and the fractions containing the IgG antibodies are pooled, dialyzed against 50 mM Tris-HCl (pH 8.0), and, if necessary, stored frozen at -70°C.

5.B. Preparation of the column

An appropriate amount of CNBr-activated Sepharose 4B gel (1 g of
20 dried gel provides for approximately 3.5 ml of hydrated gel; gel capacity is from 5 to 10 mg coupled IgG/ml of gel) manufactured by Pharmacia (17-0430-01) is suspended in 1 mM HCl buffer and washed using a buchner by adding small quantities of 1 mM HCl buffer. The total volume of buffer is 200 ml per gram of gel.

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Purified IgG antibodies are dialyzed for 4 hours at $20 \pm 5^\circ\text{C}$ against 50 volumes of 500 mM sodium phosphate buffer (pH 7.5). The antibodies are then diluted in 500 mM phosphate buffer (pH 7.5) to a final concentration of 3 mg/ml.

- 5 IgG antibodies are mixed with the gel overnight at $5 \pm 3^\circ\text{C}$. The gel is packed into a chromatography column and is washed with 2 column volumes of 500 mM phosphate buffer (pH 7.5), and 1 column volume of 50 mM sodium phosphate buffer, containing 500 mM NaCl (pH 7.5). The gel is then transferred to a tube, mixed with 100 mM ethanolamine (pH 7.5) for 4 hours at
10 room temperature, and washed twice with 2 column volumes of PBS. The gel is then stored in 1/10,000 PBS/merthiolate. The amount of IgG antibodies coupled to the gel is determined by measuring the optical density (OD) at 280 nm of the IgG solution and the direct eluate, plus washings.

5.C. Adsorption and elution of the antigen

- 15 An antigen solution in 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, for example, the supernatant obtained in 3.E.1. or the solubilized pellet obtained in 3.E.2., after centrifugation and filtration through a $0.45\ \mu\text{m}$ membrane, is applied to a column equilibrated with 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, at a flow rate of about 10 ml/hour. The column is then washed with
20 20 volumes of 50 mM Tris-HCl (pH 8.0), 2 mM EDTA. Alternatively, adsorption can be achieved by mixing overnight at $5 \pm 3^\circ\text{C}$.

- The adsorbed gel is washed with 2 to 6 volumes of 10 mM sodium phosphate buffer (pH 6.8) and the antigen is eluted with 100 mM glycine-buffer (pH 2.5). The eluate is recovered in 3 ml fractions, to each of which is added
25 150 μl of 1 M sodium phosphate buffer (pH 8.0). Absorption is measured at

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280 nm for each fraction; those fractions containing the antigen are pooled and stored at -20°C.

EXAMPLE 6: The GHPO 1360 polypeptide is useful as a serodiagnostic tool for *H. pylori* infection

The reactivity of patient sera against *H. pylori* proteins was analyzed by immunoblot technique. Briefly, total lysate of *H. pylori* strain ORV2001 was subjected to SDS-PAGE electrophoresis (BioRad protean II system) on a 12.5% gel. Proteins were electrotransferred onto a nitrocellulose paper for immunoblot assay. After blocking, the nitrocellulose paper was incubated with patient sera (1:500 diluted in blocking buffer) for one hour at room temperature, washed, and further incubated with peroxidase-conjugated goat anti-human IgG. The positive bands were revealed by incubation with the appropriate substrates. The results showed that the *H. pylori*-positive ulcer patient sera react specifically with proteins having molecular weights between 50 and 60 kDa and about 30 to 35 kDa. To identify the nature of these proteins, the reactivities of the patient sera were analyzed by immunoblot assay against purified proteins with similar molecular weights: urease (67 kDa and 30 kDa), catalase (54 kDa), heat-shock protein B (60 kDa), and the GHPO 1360 polypeptide (32 kDa) expressed and purified as described in Example 5. All patient sera showed strong reactivity against the GHPO 1360 polypeptide, but the reactivities against other purified proteins were quite variable. These results show that the GHPO 1360 polypeptide is a useful antigen for use in diagnosis of *H. pylori* infection.

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Other embodiments are within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: MERIEUX ORAVAX SOCIETE EN NOM COLLECTIF
PASTEUR MERIEUX SERUMS ET VACCINS S.A., ET
AL.
- (ii) TITLE OF THE INVENTION: 76 kDa, 30 kDa, and 50 kDa
Helicobacter Polypeptides and
Corresponding Polynucleotide Molecules
- (iii) NUMBER OF SEQUENCES: 84
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Clark & Elbing LLP
(B) STREET: 176 Federal Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US98/-----
(B) FILING DATE: 31-MAR-98
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/834,666
(B) FILING DATE: 01-APR-1997
- (viii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/831,310
(B) FILING DATE: 01-APR-1997
- (ix) ATTORNEY/AGENT INFORMATION:
(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 06132/037WO1
- (x) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-428-0200
(B) TELEFAX: 617-428-7045
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

-78-

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2798 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 328...2451
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 328...385
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCCATTCTCC GCAACCAATC CTTTAAACCA CACCACCACC AAACGAACCA AACGAAACAA      180
AAAGCATCAA AATCAAAAAA ATGACAAAAT TTTTAAGAAA ATGACAAAAA AAAAAAAAAAC      240
GATTTTATGC TATATTACG AAATCTTGTG ATAAGATCTT ATTCTTTTAA AAGACTTATC      300
TAACCATTTT AATTTCAAGG AGAAAAC ATG AAA AAA ACC CTT TTA CTC TCT CTC      354
                               Met Lys Lys Thr Leu Leu Ser Leu
                               -15

TCT CTC TCT CTC TCG TTT TTG CTC CAC GCT GAA GAC GAC GGC TTT TAC      402
Ser Leu Ser Leu Ser Phe Leu Leu His Ala Glu Asp Asp Gly Phe Tyr
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ACA AGC GTG GGC TAT CAA ATC GGT GAA GCC GCT CAA ATG GTG AAA AAC      450
Thr Ser Val Gly Tyr Gln Ile Gly Glu Ala Ala Gln Met Val Lys Asn
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ACC AAA GGC ATT CAA GAG CTT TCA GAC AAT TAT GAA AAG CTG AAC AAT      498
Thr Lys Gly Ile Gln Glu Leu Ser Asp Asn Tyr Glu Lys Leu Asn Asn
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CTT TTG AAT AAT TAC AGC ACC CTA AAC ACC CTT ATC AAA TTG TCC GCT      546
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Arg Asn Leu Leu Asp Val Lys Thr Asn Ser Pro Ala Tyr Gln Ala Val
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Thr	Ala	Asn	Gly	Ala	Asn	Gly	Asp	Gly	Val	Pro	Val	Leu	Ser	Asn	Thr		
			170					175					180				
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Thr	Thr	Lys	Leu	Asp	Phe	Thr	Ile	Asn	Gly	Asp	Lys	Arg	Thr	Gly	Gly		
			185				190					195					
AAA	CCA	AAT	ACA	CCT	GAA	AAG	TTC	CCA	TGG	AGT	GAT	GGG	AAA	TAT	ATT		1026
Lys	Pro	Asn	Thr	Pro	Glu	Lys	Phe	Pro	Trp	Ser	Asp	Gly	Lys	Tyr	Ile		
			200				205					210					
CAC	ACC	CAA	TGG	ATT	AAC	ACA	ATA	GTA	ACA	CCA	ACA	GAA	ACA	AAT	ATC		1074
His	Thr	Gln	Trp	Ile	Asn	Thr	Ile	Val	Thr	Pro	Thr	Glu	Thr	Asn	Ile		
					220					225					230		
AAC	ACA	GAA	AAT	AAC	GCT	CAA	GAG	CTT	TTA	AAA	CAA	GCG	AGC	ATC	ATT		1122
Asn	Thr	Glu	Asn	Asn	Ala	Gln	Glu	Leu	Leu	Lys	Gln	Ala	Ser	Ile	Ile		
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			250					255					260				
AGT	TAT	TGG	CAA	GGG	ATA	AGC	GGC	AAT	GGG	ACA	ATG	TGC	GGG	ATG	TTT		1218
Ser	Tyr	Trp	Gln	Gly	Ile	Ser	Gly	Asn	Gly	Thr	Met	Cys	Gly	Met	Phe		
			265				270					275					
AAG	AAT	GAA	ATC	AGC	GCG	ATC	CAA	GGC	ATG	ATC	GCT	AAC	GCT	CAA	GAA		1266
Lys	Asn	Glu	Ile	Ser	Ala	Ile	Gln	Gly	Met	Ile	Ala	Asn	Ala	Gln	Glu		
			280				285				290						
GCT	GTC	GCG	CAA	AGC	AAA	ATC	GTT	AGT	GAA	AAC	GCG	CAA	AAT	CAA	AAC		1314
Ala	Val	Ala	Gln	Ser	Lys	Ile	Val	Ser	Glu	Asn	Ala	Gln	Asn	Gln	Asn		
			295			300				305				310			
AAC	TTG	GAT	ACT	GGA	AAA	CCA	TTC	AAC	CCT	TAC	ACG	GAC	GCC	AGC	TTT		1362
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CAA GCC GAA CAA GTA GTA AAA AAC TTT GAA AAA ATC CCT ACA GCC TTT Gln Ala Glu Gln Val Val Lys Asn Phe Glu Lys Ile Pro Thr Ala Phe 345 350 355			1458
GTA TCA GAC TCT TTA GGG GTG TGT TAT GAA GTG CAA GGG GGT GAG CGT Val Ser Asp Ser Leu Gly Val Cys Tyr Glu Val Gln Gly Gly Glu Arg 360 365 370			1506
AGG GGC ACC AAT CCA GGT CAG GTA ACT TCT AAC ACT TGG GGA GCC GGT Arg Gly Thr Asn Pro Gly Gln Val Thr Ser Asn Thr Trp Gly Ala Gly 375 380 385 390			1554
TGC GCG TAT GTG AAA CAA ACC ATA ACG AAT TTA GAC AAC AGC ATC GCT Cys Ala Tyr Val Lys Gln Thr Ile Thr Asn Leu Asp Asn Ser Ile Ala 395 400 405			1602
CAC TTT GGC ACT CAA GAG CAG CAG ATA CAG CAA GCC GAA AAC ATC GCT His Phe Gly Thr Gln Glu Gln Gln Ile Gln Gln Ala Glu Asn Ile Ala 410 415 420			1650
GAC ACT CTA GTG AAT TTC AAA TCT AGA TAC AGC GAA TTA GGC AAC ACC Asp Thr Leu Val Asn Phe Lys Ser Arg Tyr Ser Glu Leu Gly Asn Thr 425 430 435			1698
TAT AAC AGC ATC ACC ACC GCG CTC TCC AAA GTC CCT AAC GCG CAA AGC Tyr Asn Ser Ile Thr Thr Ala Leu Ser Lys Val Pro Asn Ala Gln Ser 440 445 450			1746
TTG CAA AAC GTG GTG AGC AAA AAG AAT AAC CCC TAT AGC CCT CAA GGC Leu Gln Asn Val Val Ser Lys Lys Asn Asn Pro Tyr Ser Pro Gln Gly 455 460 465 470			1794
ATA GAG ACC AAT TAC TAC CTC AAT CAA AAT TCT TAC AAC CAA ATC CAA Ile Glu Thr Asn Tyr Tyr Leu Asn Gln Asn Ser Tyr Asn Gln Ile Gln 475 480 485			1842
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AAC AAG CTT TCC GTG GGG CTT TTT GGA GGG ATT GCG TTA GCG GGC ACT	2178
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Gly Val Arg Met Asn Leu Ala Arg Ser Lys Lys Lys Gly Ser Asp His	
635 640 645	
GCG GCT CAG CAT GGG ATT GAA CTA GGG CTT AAA ATC CCC ACC ATC AAC	2370
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650 655 660	
ACG AAC TAT TAT TCT TTC ATG GGG GCT GAA CTC AAA TAC AGA AGG CTT	2418
Thr Asn Tyr Tyr Ser Phe Met Gly Ala Glu Leu Lys Tyr Arg Arg Leu	
665 670 675	
TAT AGC GTG TAT TTG AAT TAT GTG TTC GCT TAC TAAGCTTTTT GTGAACTCC	2471
Tyr Ser Val Tyr Leu Asn Tyr Val Phe Ala Tyr	
680 685	
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TTTTTAAAGC TTTTTTTTGA ATTCTTTTTT TTGAATTCTT TGTTTTTAA GCTTTTTTAA	2591
ACCCTTTCGT TTTTAAATC CTTTTTTTAA GGGATTTCTT TTTTAAACT CTTTTTTTTT	2651
AAACTCTTTT TTTTAAACCC TCTTTTTTTA AGGGATTTCT TTTTAAAGCT TTTTGAAGT	2711
CTTTTTTTAA ATTCTTTTTT TGGGGGTTTG ATCTTTCTTT TTGCCAATCC CCACTACTTT	2771
CGCTTTTTAA TCTTTAGGTT TTATTTT	2798

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 708 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...19

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Leu His Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile
      1          5          10
Gly Glu Ala Ala Gln Met Val Lys Asn Thr Lys Gly Ile Gln Glu Leu
      15          20          25
Ser Asp Asn Tyr Glu Lys Leu Asn Asn Leu Leu Asn Asn Tyr Ser Thr
      30          35          40          45
Leu Asn Thr Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile Asn Asp
      50          55          60
Ala Arg Asp Asn Leu Gly Ser Ser Ser Arg Asn Leu Leu Asp Val Lys
      65          70          75
Thr Asn Ser Pro Ala Tyr Gln Ala Val Leu Leu Ala Leu Asn Ala Ala
      80          85          90
Val Gly Leu Trp Gln Val Thr Ser Tyr Ala Phe Thr Ala Cys Gly Pro
      95          100          105
Gly Ser Asn Glu Asn Ala Asn Gly Gly Ile Gln Thr Phe Asn Asn Val
      110          115          120          125
Pro Gly Gln Asp Thr Thr Thr Ile Thr Cys Asn Ser Tyr Tyr Glu Pro
      130          135          140
Gly His Gly Gly Pro Ile Ser Thr Ala Asn Tyr Ala Lys Ile Asn Gln
      145          150          155
Ala Tyr Gln Ile Ile Gln Lys Ala Leu Thr Ala Asn Gly Ala Asn Gly
      160          165          170
Asp Gly Val Pro Val Leu Ser Asn Thr Thr Thr Lys Leu Asp Phe Thr
      175          180          185
Ile Asn Gly Asp Lys Arg Thr Gly Gly Lys Pro Asn Thr Pro Glu Lys
      190          195          200          205
Phe Pro Trp Ser Asp Gly Lys Tyr Ile His Thr Gln Trp Ile Asn Thr
      210          215          220
Ile Val Thr Pro Thr Glu Thr Asn Ile Asn Thr Glu Asn Asn Ala Gln
      225          230          235
Glu Leu Leu Lys Gln Ala Ser Ile Ile Ile Thr Thr Leu Asn Glu Ala
      240          245          250
Cys Pro Asn Phe Gln Asn Gly Gly Arg Ser Tyr Trp Gln Gly Ile Ser
      255          260          265
Gly Asn Gly Thr Met Cys Gly Met Phe Lys Asn Glu Ile Ser Ala Ile
      270          275          280          285
Gln Gly Met Ile Ala Asn Ala Gln Glu Ala Val Ala Gln Ser Lys Ile
      290          295          300
Val Ser Glu Asn Ala Gln Asn Gln Asn Asn Leu Asp Thr Gly Lys Pro
      305          310          315
Phe Asn Pro Tyr Thr Asp Ala Ser Phe Ala Gln Ser Met Leu Lys Asn
      320          325          330
Ala Gln Ala Gln Ala Glu Ile Leu Asn Gln Ala Glu Gln Val Val Lys
      335          340          345
Asn Phe Glu Lys Ile Pro Thr Ala Phe Val Ser Asp Ser Leu Gly Val
      350          355          360          365
Cys Tyr Glu Val Gln Gly Gly Glu Arg Arg Gly Thr Asn Pro Gly Gln
      370          375          380
Val Thr Ser Asn Thr Trp Gly Ala Gly Cys Ala Tyr Val Lys Gln Thr

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385	390	395
Ile Thr Asn Leu Asp Asn Ser	Ile Ala His Phe Gly Thr	Gln Glu Gln
400	405	410
Gln Ile Gln Gln Ala Glu Asn	Ile Ala Asp Thr Leu Val	Asn Phe Lys
415	420	425
Ser Arg Tyr Ser Glu Leu Gly	Asn Thr Tyr Asn Ser	Ile Thr Thr Ala
430	435	440
Leu Ser Lys Val Pro Asn Ala	Gln Ser Leu Gln Asn Val	Val Ser Lys
450	455	460
Lys Asn Asn Pro Tyr Ser Pro	Gln Gly Ile Glu Thr Asn	Tyr Tyr Leu
465	470	475
Asn Gln Asn Ser Tyr Asn Gln	Ile Gln Thr Ile Asn Gln	Glu Leu Gly
480	485	490
Arg Asn Pro Phe Arg Lys Val	Gly Ile Val Asn Ser Gln	Thr Asn Asn
495	500	505
Gly Ala Met Asn Gly Ile Gly	Ile Gln Val Gly Tyr Lys	Gln Phe Phe
510	515	520
Gly Gln Lys Arg Lys Trp Gly	Ala Arg Tyr Tyr Gly Phe	Phe Asp Tyr
530	535	540
Asn His Ala Phe Ile Lys Ser	Ser Phe Phe Asn Ser Ala	Ser Asp Val
545	550	555
Trp Thr Tyr Gly Phe Gly Ala	Asp Ala Leu Tyr Asn Phe	Ile Asn Asp
560	565	570
Lys Ala Thr Asn Phe Leu Gly	Lys Asn Asn Lys Leu Ser	Val Gly Leu
575	580	585
Phe Gly Gly Ile Ala Leu Ala	Gly Thr Ser Trp Leu Asn	Ser Glu Tyr
590	595	600
Val Asn Leu Ala Thr Val Asn	Asn Val Tyr Asn Ala Lys	Met Asn Val
610	615	620
Ala Asn Phe Gln Phe Leu Phe	Asn Met Gly Val Arg Met	Asn Leu Ala
625	630	635
Arg Ser Lys Lys Lys Gly Ser	Asp His Ala Ala Gln His	Gly Ile Glu
640	645	650
Leu Gly Leu Lys Ile Pro Thr	Ile Asn Thr Asn Tyr Tyr	Ser Phe Met
655	660	665
Gly Ala Glu Leu Lys Tyr Arg	Arg Leu Tyr Ser Val Tyr	Leu Asn Tyr
670	675	680
Val Phe Ala Tyr		685

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2699 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 199...2397
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
 (B) LOCATION: 199...259
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TAAAATCCAA	TTAAAAGCGT	TCAAAGGTAA	CGCAAAAAAA	CAAAAAATGA	CGCAATTTTT	60
TCAAAATGAC	AAAAAAAAC	GCTTTATGCT	ATAATACCCC	AAATACATTC	TAATAGCAAA	120
TGCGTTCTAA	TGCAAATGCA	TTCCAATGTA	TGAAATCCCT	AATACTAAAT	CCAATTTAAT	180
CCAAAAAGGA	GAAAAAAC	ATG AAA AAA	CAC ATC CTT	TCA TTA GCT	TTA GGC	231
	Met Lys Lys	His Ile Leu	Ser Leu Ala	Leu Gly		
	-20		-15		-10	
TCG CTT TTA	GTT TCC ACT	TTG AGC GCT	GAA GAC GAC	GGC TTT TAC	ACA	279
Ser Leu Leu	Val Ser Thr	Leu Ser Ala	Glu Asp Asp	Gly Phe Tyr	Thr	
	-5		1		5	
AGC GTA GGC	TAT CAG ATC	GGT GAA GCC	GCT CAA ATG	GTA ACA AAC	ACC	327
Ser Val Gly	Tyr Gln Ile	Gly Glu Ala	Ala Gln Met	Val Thr Asn	Thr	
	10		15		20	
AAA GGC ATC	CAA CAG CTT	TCA GAC AAT	TAT GAA AAT	TTG AAC AAC	CTT	375
Lys Gly Ile	Gln Gln Leu	Ser Asp Asn	Tyr Glu Asn	Leu Asn Asn	Leu	
	25		30		35	
TTA ACG AGA	TAC AGC ACC	CTA AAC ACC	CTT ATC AAA	TTG TCC GCT	GAT	423
Leu Thr Arg	Tyr Ser Thr	Leu Asn Thr	Leu Ile Lys	Leu Ser Ala	Asp	
	40		45		50	
CCG AGC GCA	ATT AAT GCG	GTG CGG GAA	AAT CTG GGC	GCG AGC GCG	AAG	471
Pro Ser Ala	Ile Asn Ala	Val Arg Glu	Asn Leu Gly	Ala Ser Ala	Lys	
	60		65		70	
AAT TTG ATC	GGC GAT AAA	GCC AAC TCC	CCC GCC TAT	CAA GCC GTG	CTT	519
Asn Leu Ile	Gly Asp Lys	Ala Asn Ser	Pro Ala Tyr	Gln Ala Val	Leu	
	75		80		85	
TTA GCG ATC	AAC GCG GCG	GTA GGG TTT	TGG AAT GTC	GTG GGC TAT	GTG	567
Leu Ala Ile	Asn Ala Ala	Val Gly Phe	Trp Asn Val	Val Gly Tyr	Val	
	90		95		100	
ACG CAA TGT	GGG GGT AAC	GCC AAT GGT	CAA GAA AGC	ACC TCT TCA	ACC	615
Thr Gln Cys	Gly Gly Asn	Ala Asn Gly	Gln Glu Ser	Thr Ser Ser	Thr	
	105		110		115	
ACC ATC TTC	AAC AAC GAG	CCA GGG TAT	CGA TCC ACT	TCC ATC ACT	TGT	663
Thr Ile Phe	Asn Asn Glu	Pro Gly Tyr	Arg Ser Thr	Ser Ile Thr	Cys	
	120		125		130	
TCT TTG AAC	GGG CAT AAG	CCT GGA TAC	TAT GGC CCT	ATG AGC ATT	GAG	711
Ser Leu Asn	Gly His Lys	Pro Gly Tyr	Tyr Gly Pro	Met Ser Ile	Glu	
	140		145		150	
AAT TTT AAA	AAG CTT AAC	GAA GCC TAT	CAG ATC CTC	CAA ACG GCT	TTA	759
Asn Phe Lys	Lys Leu Asn	Glu Ala Tyr	Gln Ile Leu	Gln Thr Ala	Leu	

155	160	165	
AAA AAC GGC TTA CCC GCG CTC	AAA GAA AAC AAC GGG AAG	GTC AGT GTA	807
Lys Asn Gly Leu Pro Ala Leu	Lys Glu Asn Asn Gly Lys	Val Ser Val	
170	175	180	
ACC TAT ACC TAC ACA TGC TCA GGG CAA GGG AAT AAT AAC TGC TCG CCA			855
Thr Tyr Thr Tyr Thr Cys Ser Gly Gln Gly Asn Asn Asn Cys Ser Pro			
185	190	195	
AGT GTC AAC GGA ACC AAA ACC ACA ACC CAA ACC ATA GAC GGC AAA AGC			903
Ser Val Asn Gly Thr Lys Thr Thr Thr Gln Thr Ile Asp Gly Lys Ser			
200	205	210	215
GTA ACC ACC ACG ATC AGT TCA AAA GTG GTT GGT AGC ATC GCT AGT GGC			951
Val Thr Thr Thr Ile Ser Ser Lys Val Val Gly Ser Ile Ala Ser Gly			
220	225	230	
AAC ACA TCA CAT GTC ATC ACC AAC AAA TTA GAC GGT GTG CCT GAT AGC			999
Asn Thr Ser His Val Ile Thr Asn Lys Leu Asp Gly Val Pro Asp Ser			
235	240	245	
GCT CAA GCG CTC TTA GCG CAA GCG AGC ACG CTC ATC AAC ACC ATC AAC			1047
Ala Gln Ala Leu Leu Ala Gln Ala Ser Thr Leu Ile Asn Thr Ile Asn			
250	255	260	
GAA GCA TGC CCG TAT TTC CAT GCT ACT AAT AGT AGT GAG GCT AAC GCC			1095
Glu Ala Cys Pro Tyr Phe His Ala Thr Asn Ser Ser Glu Ala Asn Ala			
265	270	275	
CCA AAA TTC TCT ACT ACT ACT GGG AAA ATA TGC GGC GCT TTT TCA GAA			1143
Pro Lys Phe Ser Thr Thr Thr Gly Lys Ile Cys Gly Ala Phe Ser Glu			
280	285	290	295
GAA ATC AGC GCG ATC CAA AAG ATG ATC ACG GAC GCG CAA GAG CTA GTT			1191
Glu Ile Ser Ala Ile Gln Lys Met Ile Thr Asp Ala Gln Glu Leu Val			
300	305	310	
AAT CAA ACG AGC GTC ATT AAC AGC AAC GAA CAA TCA ACT CCG GTA GGC			1239
Asn Gln Thr Ser Val Ile Asn Ser Asn Glu Gln Ser Thr Pro Val Gly			
315	320	325	
AAT AAT AAT GGC AAG CCT TTC AAC CCT TTC ACG GAC GCA AGT TTT GCG			1287
Asn Asn Asn Gly Lys Pro Phe Asn Pro Phe Thr Asp Ala Ser Phe Ala			
330	335	340	
CAA GGC ATG CTC GCT AAC GCT AGC GCG CAA GCT AAA ATG CTC AAT TTA			1335
Gln Gly Met Leu Ala Asn Ala Ser Ala Gln Ala Lys Met Leu Asn Leu			
345	350	355	
GCC CAT CAG GTG GGG CAA GCC ATT AAC CCA GAG AAT CTT AGC GAG AAT			1383
Ala His Gln Val Gly Gln Ala Ile Asn Pro Glu Asn Leu Ser Glu Asn			
360	365	370	375
TTT AAA AAT TTT GTT ACA GGC TTT TTA GCC ACA TGC AAT AAC AAA TCA			1431
Phe Lys Asn Phe Val Thr Gly Phe Leu Ala Thr Cys Asn Asn Lys Ser			
380	385	390	

ACA GCT GGC ACT GGT GGC ACA CAA GGT TCA GCT CCA GGC ACA GTG ACC	1479
Thr Ala Gly Thr Gly Gly Thr Gln Gly Ser Ala Pro Gly Thr Val Thr	
395 400 405	
ACT CAA ACT TTC GCT TCT GGT TGC GCG TAT GTG GAG CAA ACC CTA ACG	1527
Thr Gln Thr Phe Ala Ser Gly Cys Ala Tyr Val Glu Gln Thr Leu Thr	
410 415 420	
AAC TTA GGC AAC AGC ATC GCT CAC TTT GGC ACT CAA GAG CAG CAG ATA	1575
Asn Leu Gly Asn Ser Ile Ala His Phe Gly Thr Gln Glu Gln Gln Ile	
425 430 435	
CAG CAA GCC GAA AAC ATC GCT GAC ACT CTA GTG AAT TTC AAA TCT AGA	1623
Gln Gln Ala Glu Asn Ile Ala Asp Thr Leu Val Asn Phe Lys Ser Arg	
440 445 450 455	
TAC AGC GAA TTA GGC AAC ACC TAT AAC AGC ATC ACC ACC GCG CTC TCC	1671
Tyr Ser Glu Leu Gly Asn Thr Tyr Asn Ser Ile Thr Thr Ala Leu Ser	
460 465 470	
AAA GTC CCT AAC GCG CAA AGC TTG CAA AAC GTG GTG AGC AAA AAG AAT	1719
Lys Val Pro Asn Ala Gln Ser Leu Gln Asn Val Val Ser Lys Lys Asn	
475 480 485	
AAC CCC TAT AGC CCT CAA GGC ATA GAG ACC AAT TAC TAC CTC AAT CAA	1767
Asn Pro Tyr Ser Pro Gln Gly Ile Glu Thr Asn Tyr Tyr Leu Asn Gln	
490 495 500	
AAT TCT TAC AAC CAA ATC CAA ACC ATC AAC CAA GAA CTA GGG CGT AAC	1815
Asn Ser Tyr Asn Gln Ile Gln Thr Ile Asn Gln Glu Leu Gly Arg Asn	
505 510 515	
CCC TTT AGG AAA GTG GGC ATC GTC AAT TCT CAA ACC AAC AAT GGT GCC	1863
Pro Phe Arg Lys Val Gly Ile Val Asn Ser Gln Thr Asn Asn Gly Ala	
520 525 530 535	
ATG AAT GGG ATC GGT ATT CAG GTG GGC TAT AAG CAA TTC TTT GGC CAA	1911
Met Asn Gly Ile Gly Ile Gln Val Gly Tyr Lys Gln Phe Phe Gly Gln	
540 545 550	
AAA AGA AAA TGG GGC GCT AGG TAT TAC GGC TTT TTT GAT TAC AAC CAT	1959
Lys Arg Lys Trp Gly Ala Arg Tyr Tyr Gly Phe Phe Asp Tyr Asn His	
555 560 565	
GCG TTC ATC AAA TCC AGC TTT TTC AAC TCG GCT TCT GAC GTG TGG ACT	2007
Ala Phe Ile Lys Ser Ser Phe Phe Asn Ser Ala Ser Asp Val Trp Thr	
570 575 580	
TAT GGT TTT GGA GCG GAC GCG CTT TAT AAC TTC ATC AAC GAT AAA GCC	2055
Tyr Gly Phe Gly Ala Asp Ala Leu Tyr Asn Phe Ile Asn Asp Lys Ala	
585 590 595	
ACC AAT TTC TTA GGC AAA AAC AAC AAG CTT TCT TTG GGG CTT TTT GGC	2103
Thr Asn Phe Leu Gly Lys Asn Asn Lys Leu Ser Leu Gly Leu Phe Gly	
600 605 610 615	
GGG ATT GCG TTA GCG GGC ACT TCA TGG CTC AAT TCT GAG TAC GTG AAT	2151

Gly Ile Ala Leu Ala Gly Thr Ser Trp Leu Asn Ser Glu Tyr Val Asn
620 625 630

TTA GCC ACC GTG AAT AAC GTC TAT AAC GCT AAA ATG AAT GTG GCG AAT 2199
Leu Ala Thr Val Asn Asn Val Tyr Asn Ala Lys Met Asn Val Ala Asn
635 640 645

TTC CAA TTC TTA TTC AAT ATG GGA GTG AGG ATG AAT TTA GCC AGA TCC 2247
Phe Gln Phe Leu Phe Asn Met Gly Val Arg Met Asn Leu Ala Arg Ser
650 655 660

AAG AAA AAA GGC AGC GAT CAT GCA GCT CAG CAT GGG ATT GAG TTA GGG 2295
Lys Lys Lys Gly Ser Asp His Ala Ala Gln His Gly Ile Glu Leu Gly
665 670 675

CTT AAA ATC CCC ACC ATC AAC ACG AAC TAT TAT TCC TTT ATG GGG GCT 2343
Leu Lys Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Phe Met Gly Ala
680 685 690 695

GAA CTC AAA TAC AGA AGG CTC TAT AGC GTG TAT TTG AAC TAT GTG TTC 2391
Glu Leu Lys Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn Tyr Val Phe
700 705 710

GCT TAC TAATGTTTGG CTCTTTGTGA AACTCCCTTT TTAAGGGGTT TTTTTTTGAA CT 2449
Ala Tyr

CTCTTTTAA ATTCTCTTTT TAAAGAGATT TCTTTT TTTT AAGCTTTT TTGAATTCTT 2509
TTTTTTGAA TTCTTTGTTT TTAAGCTTTT TTAAACCCT TTCGTTTAA AACTCCCTTT 2569
TTTAAGGGAT TTCTTTT TTAAGCTTTT TTTGAACCC TTTTTTTAA ACCCTCTTT 2629
TTTAAGGGGT TTCTTTTAA AGCTTTT TTAAGCTTTT TTAAATTCTT TTTTGGGGG 2689
TTTGATCTTT 2699

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 733 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...20
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Lys Lys His Ile Leu Ser Leu Ala Leu Gly Ser Leu Leu Val Ser
-20 -15 -10 -5
Thr Leu Ser Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln
1 5 10

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Ile Gly Glu Ala Ala Gln Met Val Thr Asn Thr Lys Gly Ile Gln Gln
      15                20                25
Leu Ser Asp Asn Tyr Glu Asn Leu Asn Asn Leu Leu Thr Arg Tyr Ser
      30                35                40
Thr Leu Asn Thr Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile Asn
      45                50                55                60
Ala Val Arg Glu Asn Leu Gly Ala Ser Ala Lys Asn Leu Ile Gly Asp
      65                70                75
Lys Ala Asn Ser Pro Ala Tyr Gln Ala Val Leu Leu Ala Ile Asn Ala
      80                85                90
Ala Val Gly Phe Trp Asn Val Val Gly Tyr Val Thr Gln Cys Gly Gly
      95                100                105
Asn Ala Asn Gly Gln Glu Ser Thr Ser Ser Thr Thr Ile Phe Asn Asn
      110                115                120
Glu Pro Gly Tyr Arg Ser Thr Ser Ile Thr Cys Ser Leu Asn Gly His
      125                130                135                140
Lys Pro Gly Tyr Tyr Gly Pro Met Ser Ile Glu Asn Phe Lys Lys Leu
      145                150                155
Asn Glu Ala Tyr Gln Ile Leu Gln Thr Ala Leu Lys Asn Gly Leu Pro
      160                165                170
Ala Leu Lys Glu Asn Asn Gly Lys Val Ser Val Thr Tyr Thr Tyr Thr
      175                180                185
Cys Ser Gly Gln Gly Asn Asn Asn Cys Ser Pro Ser Val Asn Gly Thr
      190                195                200
Lys Thr Thr Thr Gln Thr Ile Asp Gly Lys Ser Val Thr Thr Thr Ile
      205                210                215                220
Ser Ser Lys Val Val Gly Ser Ile Ala Ser Gly Asn Thr Ser His Val
      225                230                235
Ile Thr Asn Lys Leu Asp Gly Val Pro Asp Ser Ala Gln Ala Leu Leu
      240                245                250
Ala Gln Ala Ser Thr Leu Ile Asn Thr Ile Asn Glu Ala Cys Pro Tyr
      255                260                265
Phe His Ala Thr Asn Ser Ser Glu Ala Asn Ala Pro Lys Phe Ser Thr
      270                275                280
Thr Thr Gly Lys Ile Cys Gly Ala Phe Ser Glu Glu Ile Ser Ala Ile
      285                290                295                300
Gln Lys Met Ile Thr Asp Ala Gln Glu Leu Val Asn Gln Thr Ser Val
      305                310                315
Ile Asn Ser Asn Glu Gln Ser Thr Pro Val Gly Asn Asn Asn Gly Lys
      320                325                330
Pro Phe Asn Pro Phe Thr Asp Ala Ser Phe Ala Gln Gly Met Leu Ala
      335                340                345
Asn Ala Ser Ala Gln Ala Lys Met Leu Asn Leu Ala His Gln Val Gly
      350                355                360
Gln Ala Ile Asn Pro Glu Asn Leu Ser Glu Asn Phe Lys Asn Phe Val
      365                370                375                380
Thr Gly Phe Leu Ala Thr Cys Asn Asn Lys Ser Thr Ala Gly Thr Gly
      385                390                395
Gly Thr Gln Gly Ser Ala Pro Gly Thr Val Thr Thr Gln Thr Phe Ala
      400                405                410
Ser Gly Cys Ala Tyr Val Glu Gln Thr Leu Thr Asn Leu Gly Asn Ser
      415                420                425
Ile Ala His Phe Gly Thr Gln Glu Gln Gln Ile Gln Gln Ala Glu Asn
      430                435                440
Ile Ala Asp Thr Leu Val Asn Phe Lys Ser Arg Tyr Ser Glu Leu Gly
      445                450                455                460
Asn Thr Tyr Asn Ser Ile Thr Thr Ala Leu Ser Lys Val Pro Asn Ala

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465										470					475				
Gln	Ser	Leu	Gln	Asn	Val	Val	Ser	Lys	Lys	Asn	Asn	Pro	Tyr	Ser	Pro				
480										485					490				
Gln	Gly	Ile	Glu	Thr	Asn	Tyr	Tyr	Leu	Asn	Gln	Asn	Ser	Tyr	Asn	Gln				
495										500					505				
Ile	Gln	Thr	Ile	Asn	Gln	Glu	Leu	Gly	Arg	Asn	Pro	Phe	Arg	Lys	Val				
510										515					520				
Gly	Ile	Val	Asn	Ser	Gln	Thr	Asn	Asn	Gly	Ala	Met	Asn	Gly	Ile	Gly				
525										530					535				
Ile	Gln	Val	Gly	Tyr	Lys	Gln	Phe	Phe	Gly	Gln	Lys	Arg	Lys	Trp	Gly				
545										550					555				
Ala	Arg	Tyr	Tyr	Gly	Phe	Phe	Asp	Tyr	Asn	His	Ala	Phe	Ile	Lys	Ser				
560										565					570				
Ser	Phe	Phe	Asn	Ser	Ala	Ser	Asp	Val	Trp	Thr	Tyr	Gly	Phe	Gly	Ala				
575										580					585				
Asp	Ala	Leu	Tyr	Asn	Phe	Ile	Asn	Asp	Lys	Ala	Thr	Asn	Phe	Leu	Gly				
590										595					600				
Lys	Asn	Asn	Lys	Leu	Ser	Leu	Gly	Leu	Phe	Gly	Gly	Ile	Ala	Leu	Ala				
605										610					615				
Gly	Thr	Ser	Trp	Leu	Asn	Ser	Glu	Tyr	Val	Asn	Leu	Ala	Thr	Val	Asn				
625										630					635				
Asn	Val	Tyr	Asn	Ala	Lys	Met	Asn	Val	Ala	Asn	Phe	Gln	Phe	Leu	Phe				
640										645					650				
Asn	Met	Gly	Val	Arg	Met	Asn	Leu	Ala	Arg	Ser	Lys	Lys	Lys	Gly	Ser				
655										660					665				
Asp	His	Ala	Ala	Gln	His	Gly	Ile	Glu	Leu	Gly	Leu	Lys	Ile	Pro	Thr				
670										675					680				
Ile	Asn	Thr	Asn	Tyr	Tyr	Ser	Phe	Met	Gly	Ala	Glu	Leu	Lys	Tyr	Arg				
685										690					695				
Arg	Leu	Tyr	Ser	Val	Tyr	Leu	Asn	Tyr	Val	Phe	Ala	Tyr							
705										710									

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2915 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
(B) LOCATION: 365...2597
(D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
(B) LOCATION: 365...425
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTTTAGGCGA CAAAATCGCT TATGTTGGGG ATAAAGGCAA CCCGCACAAT TTCGCTCACA	60
AGAAATAAAC CGCTCATAAG GGGCAAACGC CCCAAAAAAG CGATTTTAA AGAGGTTACG	120
GCAAAATCAA GCTCTTTAGT ATTTAATCTT AAAAAATGCT AAAAGCCTTT TTATGGGCTA	180
ACACCACACA AAAAGCATCA AAATCAAAAA AATGACAAAA TTTTAAAGAA AATGACAAAA	240
AAAAACGCTT TATGCTATAA TACCCCAAAT ACATTCTAAT AGCAAATGCG TTCTAATGCA	300
AATGCATTCC AATGTATGAA ATCCCTAATA CTAAATCCAA TTTAATCCAA AAAGGAGAAA	360
AAAC ATG AAA AAA CAC ATC CTT TCA TTA GCT TTA GGC TCG CTT TTA GTT	409
Met Lys Lys His Ile Leu Ser Leu Ala Leu Gly Ser Leu Leu Val	
-20 -15 -10	
TCC ACT TTG AGC GCT GAA GAC GAC GGC TTT TAC ACA AGC GTA GGC TAT	457
Ser Thr Leu Ser Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr	
-5 1 5 10	
CAG ATC GGT GAA GCC GCT CAA ATG GTA ACA AAC ACC AAA GGC ATC CAA	505
Gln Ile Gly Glu Ala Ala Gln Met Val Thr Asn Thr Lys Gly Ile Gln	
15 20 25	
CAG CTT TCA GAC AAT TAT GAA AAT TTG AAC AAC CTT TTA ACG AGA TAC	553
Gln Leu Ser Asp Asn Tyr Glu Asn Leu Asn Asn Leu Leu Thr Arg Tyr	
30 35 40	
AGC ACC CTA AAC ACC CTT ATC AAA TTG TCC GCT GAT CCG AGC GCA ATT	601
Ser Thr Leu Asn Thr Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile	
45 50 55	
AAT GCG GTG CGG GAA AAT CTG GGC GCG AGC ACG AAG AAT TTG ATC GGC	649
Asn Ala Val Arg Glu Asn Leu Gly Ala Ser Thr Lys Asn Leu Ile Gly	
60 65 70 75	
GAT AAA GCC AAC TCC CCG GCG TAT CAA GCC GTG TTT TTA GCG ATC AAC	697
Asp Lys Ala Asn Ser Pro Ala Tyr Gln Ala Val Phe Leu Ala Ile Asn	
80 85 90	
GCG GCG GTA GGG TTG TGG AAT ACC ATC GGC TAT GCG GTC ATG TGC GGG	745
Ala Ala Val Gly Leu Trp Asn Thr Ile Gly Tyr Ala Val Met Cys Gly	
95 100 105	
AAC GGG AAC GGC ACA GAG AGT GGG CCT GGC AGC GTG ATC TTT AAT GAC	793
Asn Gly Asn Gly Thr Glu Ser Gly Pro Gly Ser Val Ile Phe Asn Asp	
110 115 120	
CAA CCA GGA CAG GAT TCC ACG CAA ATT ACT TGC AAC CGC TTT GAA TCA	841
Gln Pro Gly Gln Asp Ser Thr Gln Ile Thr Cys Asn Arg Phe Glu Ser	
125 130 135	
ACT GGG CCT GGT AAA AGC ATG TCT ATT GAT GAA TTC AAA AAA CTC AAT	889
Thr Gly Pro Gly Lys Ser Met Ser Ile Asp Glu Phe Lys Lys Leu Asn	
140 145 150 155	
GAA GCC TAT CAA ATC ATC CAG CAA GCT TTA AAA AAT CAA AGT GGG TTT	937
Glu Ala Tyr Gln Ile Ile Gln Gln Ala Leu Lys Asn Gln Ser Gly Phe	
160 165 170	
CCT GAA TTA GGC GGG AAC GGC ACA AAA GTG AGT GTT AAT TAC AAT TAC	985
Pro Glu Leu Gly Gly Asn Gly Thr Lys Val Ser Val Asn Tyr Asn Tyr	
175 180 185	

GAA TGC AGA CAA ACT GCT GAT ATC AAC GGC GGT GTG TAT CAG TTC TGC	1033
Glu Cys Arg Gln Thr Ala Asp Ile Asn Gly Gly Val Tyr Gln Phe Cys	
190 195 200	
AAG GCT AAA AAT GGT AGT AGT AGC AGT AGT AAT GGC GGT AAT GGC AGT	1081
Lys Ala Lys Asn Gly Ser Ser Ser Ser Ser Asn Gly Gly Asn Gly Ser	
205 210 215	
AGC ACG CAA ACA ACC GCG ACA ACC ACG CAA GAC GGC GTA ACG ATC ACC	1129
Ser Thr Gln Thr Thr Ala Thr Thr Thr Gln Asp Gly Val Thr Ile Thr	
220 225 230 235	
ACT ACC TAT AAT AAT AAC AAA GCC ACC GTC AAA TTT GAC ATC ACC AAT	1177
Thr Thr Tyr Asn Asn Asn Lys Ala Thr Val Lys Phe Asp Ile Thr Asn	
240 245 250	
AAC GCT GAA CAG CTG TTA AAT CAA GCG GCA AAC ATC ATG CAA GTC CTT	1225
Asn Ala Glu Gln Leu Leu Asn Gln Ala Ala Asn Ile Met Gln Val Leu	
255 260 265	
AAT ACG CAA TGC CCT TTA GTG CGT TCC ACG AAT AAC GAA AAC ACT CCA	1273
Asn Thr Gln Cys Pro Leu Val Arg Ser Thr Asn Asn Glu Asn Thr Pro	
270 275 280	
GGG GGT GGT CAA CCA TGG GGT TTA AGC ACA TCC GGG AAT GCG TGC AGC	1321
Gly Gly Gly Gln Pro Trp Gly Leu Ser Thr Ser Gly Asn Ala Cys Ser	
285 290 295	
ATC TTC CAA CAA GAA TTT AGC CAG GTT ACT AGC ATG ATC AAA AAC GCC	1369
Ile Phe Gln Gln Glu Phe Ser Gln Val Thr Ser Met Ile Lys Asn Ala	
300 305 310 315	
CAA GAA ATA ATC GCG CAA AGC AAA ATC GTT AGT GAA AAC GCG CAA AAT	1417
Gln Glu Ile Ile Ala Gln Ser Lys Ile Val Ser Glu Asn Ala Gln Asn	
320 325 330	
CAA AAC AAC TTG GAT ACT GGA AAA CCA TTC AAC CCT TAC ACG GAC GCC	1465
Gln Asn Asn Leu Asp Thr Gly Lys Pro Phe Asn Pro Tyr Thr Asp Ala	
335 340 345	
AGC TTT GCG CAA AGC ATG CTC AAA AAC GCT CAA GCG CAA GCA GAG ATG	1513
Ser Phe Ala Gln Ser Met Leu Lys Asn Ala Gln Ala Gln Ala Glu Met	
350 355 360	
TTC AAT TTG AGC GAA CAA GTG AAA AAG AAC TTG GAA GTC ATG AAA AAC	1561
Phe Asn Leu Ser Glu Gln Val Lys Lys Asn Leu Glu Val Met Lys Asn	
365 370 375	
AAC AAT AAT GTT AAC GAG AAA TTA GCA GGA TTT GGG AAA GAA GAA GTA	1609
Asn Asn Asn Val Asn Glu Lys Leu Ala Gly Phe Gly Lys Glu Glu Val	
380 385 390 395	
ATG ACC AAT TTT GTT AGC GCC TTT TTG GCA AGC TGC AAA GAT GGT GGC	1657
Met Thr Asn Phe Val Ser Ala Phe Leu Ala Ser Cys Lys Asp Gly Gly	
400 405 410	
ACA TTG CCT AAT GCA GGG GTT ACT TCT AAC ACT TGG GGG GCG GGT TGC	1705

Thr Leu Pro	Asn Ala Gly Val Thr Ser Asn Thr Trp Gly Ala Gly Cys	
415	420	425
GCG TAT GTG GGA GAG ACG ATA AGC GCC CTA ACC AAC AGC ATC GCT CAC	1753	
Ala Tyr Val Gly Glu Thr Ile Ser Ala Leu Thr Asn Ser Ile Ala His		
430	435	440
TTT GGC ACT CAA GAG CAG CAG ATA CAG CAA GCC GAA AAC ATC GCT GAC	1801	
Phe Gly Thr Gln Glu Gln Gln Ile Gln Gln Ala Glu Asn Ile Ala Asp		
445	450	455
ACT CTA GTG AAT TTC AAA TCT AGA TAC AGC GAA TTA GGC AAC ACC TAT	1849	
Thr Leu Val Asn Phe Lys Ser Arg Tyr Ser Glu Leu Gly Asn Thr Tyr		
460	465	470
AAC AGC ATC ACC ACC GCG CTC TCC AAA GTC CCT AAC GCG CAA AGC TTG	1897	
Asn Ser Ile Thr Thr Ala Leu Ser Lys Val Pro Asn Ala Gln Ser Leu		
480	485	490
CAA AAC GTG GTG AGC AAA AAG AAT AAC CCC TAT AGC CCT CAA GGC ATA	1945	
Gln Asn Val Val Ser Lys Lys Asn Asn Pro Tyr Ser Pro Gln Gly Ile		
495	500	505
GAG ACC AAT TAC TAC CTC AAT CAA AAT TCT TAC AAC CAA ATC CAA ACC	1993	
Glu Thr Asn Tyr Tyr Leu Asn Gln Asn Ser Tyr Asn Gln Ile Gln Thr		
510	515	520
ATC AAC CAA GAA CTA GGG CGT AAC CCC TTT AGG AAA GTG GGC ATC GTC	2041	
Ile Asn Gln Glu Leu Gly Arg Asn Pro Phe Arg Lys Val Gly Ile Val		
525	530	535
AAT TCT CAA ACC AAC AAT GGT GCC ATG AAT GGG ATC GGC ATT CAG GTG	2089	
Asn Ser Gln Thr Asn Asn Gly Ala Met Asn Gly Ile Gly Ile Gln Val		
540	545	550
GGC TAT AAG CAA TTC TTT GGC CAA AAA AGA AAA TGG GGC GCT AGG TAT	2137	
Gly Tyr Lys Gln Phe Phe Gly Gln Lys Arg Lys Trp Gly Ala Arg Tyr		
560	565	570
TAC GGC TTT TTT GAT TAC AAC CAT GCG TTC ATC AAA TCC AGC TTT TTC	2185	
Tyr Gly Phe Phe Asp Tyr Asn His Ala Phe Ile Lys Ser Ser Phe Phe		
575	580	585
AAC TCG GCT TCT GAC GTG TGG ACT TAT GGT TTT GGA GCG GAC GCG CTT	2233	
Asn Ser Ala Ser Asp Val Trp Thr Tyr Gly Phe Gly Ala Asp Ala Leu		
590	595	600
TAT AAC TTC ATC AAC GAT AAA GCC ACC AAT TTC TTA GGC AAA AAC AAC	2281	
Tyr Asn Phe Ile Asn Asp Lys Ala Thr Asn Phe Leu Gly Lys Asn Asn		
605	610	615
AAG CTT TCT TTG GGG CTT TTT GGC GGG ATT GCG TTA GCG GGC ACT TCA	2329	
Lys Leu Ser Leu Gly Leu Phe Gly Gly Ile Ala Leu Ala Gly Thr Ser		
620	625	630
TGG CTC AAT TCT GAG TAC GTG AAT TTA GCC ACC GTG AAT AAC GTC TAT	2377	
Trp Leu Asn Ser Glu Tyr Val Asn Leu Ala Thr Val Asn Asn Val Tyr		

640	645	650	
AAC GCT AAA ATG AAT GTG GCG AAT TTC CAA TTC TTA TTC AAT ATG GGA			2425
Asn Ala Lys Met Asn Val Ala Asn Phe Gln Phe Leu Phe Asn Met Gly			
655	660	665	
GTG AGG ATG AAT TTA GCC AGA TCC AAG AAA AAA GGC AGC GAT CAT GCA			2473
Val Arg Met Asn Leu Ala Arg Ser Lys Lys Lys Gly Ser Asp His Ala			
670	675	680	
GCT CAG CAT GGG ATT GAG TTA GGG CTT AAA ATC CCC ACC ATC AAC ACG			2521
Ala Gln His Gly Ile Glu Leu Gly Leu Lys Ile Pro Thr Ile Asn Thr			
685	690	695	
AAC TAT TAT TCC TTT ATG GGG GCT GAA CTC AAA TAC AGA AGG CTC TAT			2569
Asn Tyr Tyr Ser Phe Met Gly Ala Glu Leu Lys Tyr Arg Arg Leu Tyr			
700	705	710	715
AGC GTG TAT TTG AAT NAT GTG TTC GCT TAC TAAGCTTTTT GTGAAACTCC			2619
Ser Val Tyr Leu Asn Xaa Val Phe Ala Tyr			
720	725		
CTTTTAAAGG GGTTTTTTTT TGAACCTCTCT TTTAAATTCT CTTTTTAAAG AGATTTCTTT			2679
TTTTAAGCTT TTTTTTGAAC TTTTTTTTGA ATTCTTTGTT TTTAAGCTTT TTTTAAACCC			2739
TTTCGTTTTT AAACCTCCCTT TTTTAAGGGA TTTCTTTTTT TGAACCTCCT TTTTGAACC			2799
CTTTTTTTTA AACCTCTTTT TTTTAAGGGG TTTCTTTTTA AAGCTTTTTT GAAGTCTTTT			2859
TTTAAATTCT TTTTTTGGGG GTTTGATCTT TCTTTTTGCC AATCCCCACT ACTTTC			2915

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 745 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...20
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Lys	Lys	His	Ile	Leu	Ser	Leu	Ala	Leu	Gly	Ser	Leu	Leu	Val	Ser
-20					-15					-10					-5
Thr	Leu	Ser	Ala	Glu	Asp	Asp	Gly	Phe	Tyr	Thr	Ser	Val	Gly	Tyr	Gln
			1				5						10		
Ile	Gly	Glu	Ala	Ala	Gln	Met	Val	Thr	Asn	Thr	Lys	Gly	Ile	Gln	Gln
		15				20					25				
Leu	Ser	Asp	Asn	Tyr	Glu	Asn	Leu	Asn	Asn	Leu	Leu	Thr	Arg	Tyr	Ser

SUBSTITUTE SHEET (RULE 26)

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Asn Val Val Ser Lys Lys Asn Asn Pro Tyr Ser Pro Gln Gly Ile Glu
    495                                500                                505
Thr Asn Tyr Tyr Leu Asn Gln Asn Ser Tyr Asn Gln Ile Gln Thr Ile
    510                                515                                520
Asn Gln Glu Leu Gly Arg Asn Pro Phe Arg Lys Val Gly Ile Val Asn
    525                                530                                535                                540
Ser Gln Thr Asn Asn Gly Ala Met Asn Gly Ile Gly Ile Gln Val Gly
    545                                550                                555
Tyr Lys Gln Phe Phe Gly Gln Lys Arg Lys Trp Gly Ala Arg Tyr Tyr
    560                                565                                570
Gly Phe Phe Asp Tyr Asn His Ala Phe Ile Lys Ser Ser Phe Phe Asn
    575                                580                                585
Ser Ala Ser Asp Val Trp Thr Tyr Gly Phe Gly Ala Asp Ala Leu Tyr
    590                                595                                600
Asn Phe Ile Asn Asp Lys Ala Thr Asn Phe Leu Gly Lys Asn Asn Lys
    605                                610                                615                                620
Leu Ser Leu Gly Leu Phe Gly Gly Ile Ala Leu Ala Gly Thr Ser Trp
    625                                630                                635
Leu Asn Ser Glu Tyr Val Asn Leu Ala Thr Val Asn Asn Val Tyr Asn
    640                                645                                650
Ala Lys Met Asn Val Ala Asn Phe Gln Phe Leu Phe Asn Met Gly Val
    655                                660                                665
Arg Met Asn Leu Ala Arg Ser Lys Lys Lys Gly Ser Asp His Ala Ala
    670                                675                                680
Gln His Gly Ile Glu Leu Gly Leu Lys Ile Pro Thr Ile Asn Thr Asn
    685                                690                                695                                700
Tyr Tyr Ser Phe Met Gly Ala Glu Leu Lys Tyr Arg Arg Leu Tyr Ser
    705                                710                                715
Val Tyr Leu Asn Xaa Val Phe Ala Tyr
    720                                725

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2603 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 210...2342
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 210...270
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGACCTTTA TTGGTTTAAT ATTTGTTTAG AAATAACACA AAAACCTTTT TTTTTTTTTT

60

SUBSTITUTE SHEET (RULE 26)

GGA GAA AGA TGG AAA TTC CCA TGG ACT AAT GGA AAA TTT GTT TCA GTC	905
Gly Glu Arg Trp Lys Phe Pro Trp Thr Asn Gly Lys Phe Val Ser Val	
200 205 210	
AAG TGG GTG AAT GGG AAG TAT GAA GAA ATT AAA GAA GAC ATC AAA GTG	953
Lys Trp Val Asn Gly Lys Tyr Glu Glu Ile Lys Glu Asp Ile Lys Val	
215 220 225	
TCA AAT AAC GCT CAA GAG CTT TTA AAA CAG GCT AGC ACT ATT TTA ACC	1001
Ser Asn Asn Ala Gln Glu Leu Leu Lys Gln Ala Ser Thr Ile Leu Thr	
230 235 240	
ACT CTT AAT GAA GCA TGC CCA TGG TTG AGT AAT GGT GGT GCA GGC AAT	1049
Thr Leu Asn Glu Ala Cys Pro Trp Leu Ser Asn Gly Gly Ala Gly Asn	
245 250 255 260	
GTG GCC GGT GGC AAT AGT TTA TGG GCC GGA ATA GAT AAA GGC GAC GGG	1097
Val Ala Gly Gly Asn Ser Leu Trp Ala Gly Ile Asp Lys Gly Asp Gly	
265 270 275	
AGC GCA TGC GGG ATT TTT AAA AAT GAA ATC AGC GCG ATT CAA GAC ATG	1145
Ser Ala Cys Gly Ile Phe Lys Asn Glu Ile Ser Ala Ile Gln Asp Met	
280 285 290	
ATC AAA AAC GCT GAA ATA GCC GTA GAG CAA TCC AAA ATC GTT ACC GCC	1193
Ile Lys Asn Ala Glu Ile Ala Val Glu Gln Ser Lys Ile Val Thr Ala	
295 300 305	
AAC GCG CAA AAC CAG CAC AAC CTA GAC ACT GGG AAA GCA TTC AAC CCC	1241
Asn Ala Gln Asn Gln His Asn Leu Asp Thr Gly Lys Ala Phe Asn Pro	
310 315 320	
TAT AAA GAC GCC AAC TTC GCC CAA AGC ATG TTC GCT AAC GCT AGA GCG	1289
Tyr Lys Asp Ala Asn Phe Ala Gln Ser Met Phe Ala Asn Ala Arg Ala	
325 330 335 340	
CAA GCG GAG ATT TTA AAC CGC GCT CAA GCA GTG GTG AAG GAC TTT GAA	1337
Gln Ala Glu Ile Leu Asn Arg Ala Gln Ala Val Val Lys Asp Phe Glu	
345 350 355	
AGA ATC CCT GCA GCG TTC GTG AAA GAC TCT TTA GGA GTA TGC CAT GAA	1385
Arg Ile Pro Ala Ala Phe Val Lys Asp Ser Leu Gly Val Cys His Glu	
360 365 370	
AAG GGT AGC GAC GGC AAT CTC CGT GGC ACG CCA TCT GGC ACG GTT ACT	1433
Lys Gly Ser Asp Gly Asn Leu Arg Gly Thr Pro Ser Gly Thr Val Thr	
375 380 385	
TCT AAC ACT TGG GGA GCC GGC TGC GCG TAT GTG GGA GAA ACC GTA ACG	1481
Ser Asn Thr Trp Gly Ala Gly Cys Ala Tyr Val Gly Glu Thr Val Thr	
390 395 400	
AAT CTA AAA AAC AGC ATC GCT CAT TTT GGC GAC CAA GCG GAG CGA ATC	1529
Asn Leu Lys Asn Ser Ile Ala His Phe Gly Asp Gln Ala Glu Arg Ile	
405 410 415 420	
CAT AAT GCG CGA AAT CTC GCC TAC ACT TTA GCG AAT TTC AGC GGC CAG	1577

His Asn Ala Arg Asn Leu Ala Tyr Thr Leu Ala Asn Phe Ser Gly Gln	
425 430 435	
TAC AAA AAG CTA GGC GAA CAC TAT GAC AGC ATC ACA GCG GCG CTC TCT	1625
Tyr Lys Lys Leu Gly Glu His Tyr Asp Ser Ile Thr Ala Ala Leu Ser	
440 445 450	
AGC TTG CCT GAT GCG CAA TCT TTA CAA AAT GTG GTG AGC AAA AAG ACT	1673
Ser Leu Pro Asp Ala Gln Ser Leu Gln Asn Val Val Ser Lys Lys Thr	
455 460 465	
AAC CCT AAC AGC CCG CAA GGC ATA CAG GAT AAT TAC TAC ATT GAC TCC	1721
Asn Pro Asn Ser Pro Gln Gly Ile Gln Asp Asn Tyr Tyr Ile Asp Ser	
470 475 480	
AAC ATC CAT TCT CAA GTG CAA TCT AGG AGT CAA GAA CTC GGC AGT AAC	1769
Asn Ile His Ser Gln Val Gln Ser Arg Ser Gln Glu Leu Gly Ser Asn	
485 490 495 500	
CCT TTC AGA CGC GCC GGG CTA ATC GCC GCT TCT ACC ACC AAT AAC GGC	1817
Pro Phe Arg Arg Ala Gly Leu Ile Ala Ala Ser Thr Thr Asn Asn Gly	
505 510 515	
GCG ATG AAT GGG ATT GGC TTT CAA GTG GGC TAT AAG CAA TTC TTT GGG	1865
Ala Met Asn Gly Ile Gly Phe Gln Val Gly Tyr Lys Gln Phe Phe Gly	
520 525 530	
AAA AAC AAA CGA TGG GGC GCG AGA TAC TAC GGC TTT GTG GAT TAC AAC	1913
Lys Asn Lys Arg Trp Gly Ala Arg Tyr Tyr Gly Phe Val Asp Tyr Asn	
535 540 545	
CAC ACC TAT AAC AAG TCC CAA TTT TTC AAC TCC GAT TCT GAT GTT TGG	1961
His Thr Tyr Asn Lys Ser Gln Phe Phe Asn Ser Asp Ser Asp Val Trp	
550 555 560	
ACT TAT GGC GTG GGG AGC GAT TTG TTA GTG AAT TTC ATC AAC GAT AAA	2009
Thr Tyr Gly Val Gly Ser Asp Leu Leu Val Asn Phe Ile Asn Asp Lys	
565 570 575 580	
GCC ACT AAA CAC AAT AAA ATT TCT TTT GGC GCG TTT GGC GGT ATC CAA	2057
Ala Thr Lys His Asn Lys Ile Ser Phe Gly Ala Phe Gly Gly Ile Gln	
585 590 595	
CTA GCC GGG ACT TCA TGG CTT AAT TCT CAG TAT GTG AAT TTA GCG AAT	2105
Leu Ala Gly Thr Ser Trp Leu Asn Ser Gln Tyr Val Asn Leu Ala Asn	
600 605 610	
GTG AAC AAT TAT TAT AAA GCT AAA ATC AAC ACC TCT AAC TTC CAA TTC	2153
Val Asn Asn Tyr Tyr Lys Ala Lys Ile Asn Thr Ser Asn Phe Gln Phe	
615 620 625	
TTA TTC AAT CTG GGC TTA AGG ACC AAT CTC GCC AGA AAT AAA AGA ATA	2201
Leu Phe Asn Leu Gly Leu Arg Thr Asn Leu Ala Arg Asn Lys Arg Ile	
630 635 640	
GGC GCT GAT CAT AGC GCG CAA CAT GGC ATG GAA TTA GGC GTG AAG ATC	2249
Gly Ala Asp His Ser Ala Gln His Gly Met Glu Leu Gly Val Lys Ile	

645	650	655	660	
CCC ACG ATC AAC ACA AAT TAC TAT TCT TTG CTA GGC ACT ACC TTG CAA				2297
Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Leu Leu Gly Thr Thr Leu Gln				
	665	670	675	
TAC AGA AGG CTT TAT AGC GTG TAT CTC AAC TAT GTG TTT GCT TAC TAAAA				2347
Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn Tyr Val Phe Ala Tyr				
	680	685	690	
GCTTAAACTC CTTTTTAAAC TCCCTTTTAA GGGGGTTTAA TCTTTTAAAC TGACTTTTCT				2407
TTTAGCTTTT TTTAATTTTT TCCACCAAAC AAAGTTTTTT GACTTCAAGC GTTAATCACA				2467
AAAAATACTC AAAGGCGTTT TTTGCAATCT AAATAAAAAA TTAGCGTTAT TCAAGCGATC				2527
ATTTTAAACC ACCCAAGCAA GAAACCCCAA ACATCTTTAG CGTTCGCGCG CTCCACTAAC				2587
CAAAAAACGC CCCAAA				2603

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 711 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...20
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Lys	Lys	Lys	Phe	Leu	Ser	Leu	Thr	Leu	Gly	Ser	Leu	Leu	Val	Ser
-20					-15					-10					-5
Ala	Leu	Ser	Ala	Glu	Asp	Asn	Gly	Phe	Phe	Val	Ser	Ala	Gly	Tyr	Gln
			1				5						10		
Ile	Gly	Glu	Ser	Ala	Gln	Met	Val	Lys	Asn	Thr	Lys	Gly	Ile	Gln	Asp
	15					20					25				
Leu	Ser	Asp	Ser	Tyr	Glu	Arg	Leu	Asn	Asn	Leu	Leu	Thr	Ser	Tyr	Ser
	30					35					40				
Ala	Leu	Asn	Thr	Leu	Ile	Arg	Gln	Ser	Ala	Asp	Pro	Asn	Ala	Ile	Asn
45					50					55				60	
Asn	Ala	Arg	Gly	Asn	Leu	Asn	Ala	Ser	Ala	Lys	Asn	Leu	Ile	Asn	Asp
			65				70						75		
Lys	Lys	Asn	Ser	Pro	Ala	Tyr	Gln	Ala	Val	Leu	Leu	Ala	Leu	Asn	Ala
		80					85						90		
Ala	Ala	Gly	Leu	Trp	Gln	Val	Met	Ser	Tyr	Ser	Ile	Ser	Val	Cys	Gly
	95					100						105			
Pro	Gly	Ser	Asp	Lys	Asn	Lys	Asn	Gly	Gly	Val	Gln	Thr	Phe	Glu	Asn
	110				115					120					
Val	Pro	Ser	Asn	Gly	Gly	Thr	Thr	Ile	Ala	Cys	Asp	Ser	Phe	Tyr	Glu
125				130						135				140	
Pro	Gly	Lys	Trp	Ser	Gly	Ile	Ser	Thr	Glu	Asn	Tyr	Ala	Lys	Ile	Asn

[illegible]

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Ser Gln Tyr Val Asn Leu Ala Asn Val Asn Asn Tyr Tyr Lys Ala Lys
605                610                615                620
Ile Asn Thr Ser Asn Phe Gln Phe Leu Phe Asn Leu Gly Leu Arg Thr
                625                630                635
Asn Leu Ala Arg Asn Lys Arg Ile Gly Ala Asp His Ser Ala Gln His
                640                645                650
Gly Met Glu Leu Gly Val Lys Ile Pro Thr Ile Asn Thr Asn Tyr Tyr
                655                660                665
Ser Leu Leu Gly Thr Thr Leu Gln Tyr Arg Arg Leu Tyr Ser Val Tyr
                670                675                680
Leu Asn Tyr Val Phe Ala Tyr
685                690

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2427 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 232...2247
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 232...292
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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AAAACGCGCA GCAAAAAATC TCTGTTAAGC TTTTATCATT AGCGTTCCAT TGAAACAAAA      60
TCTAAAAACC CTTTCCAATA CCACCCAAAC AAACGCGCAA AAAATGCAAA AATTCTAAAT      120
TTTCTCCAAA TGACAAAAAA AAAAAAACG ATTTTATGCT ACAATGCTTT TAATACATTC      180
TTACTTAATG TATAAAATCT CAATCACTCA ATTTAATTTT AAAGGATATT T ATG AAA      237
                                     Met Lys
                                     -20

AAA ACC CTT TTA CTC TCT CTC TCT CTC TCT CTC TCG TCA TCG CTT TTA      285
Lys Thr Leu Leu Leu Ser Leu Ser Leu Ser Leu Ser Ser Ser Leu Leu
                -15                -10                -5

AAC GCT GAA GAC AAC GGC TTT TTT ATC AGC GCG GGC TAT CAA ATC GGT      333
Asn Ala Glu Asp Asn Gly Phe Phe Ile Ser Ala Gly Tyr Gln Ile Gly
                1                5                10

GAA GCC GCT CAA ATG GTG AAA AAC ACC GGC GAA TTG AAA AAA CTT TCA      381
Glu Ala Ala Gln Met Val Lys Asn Thr Gly Glu Leu Lys Lys Leu Ser
15                20                25                30

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GAC ACT TAT GAG AAT TTG AGC AAC CTT TTA ACC AAT TTT AAC AAC CTC	429
Asp Thr Tyr Glu Asn Leu Ser Asn Leu Leu Thr Asn Phe Asn Asn Leu	
35 40 45	
AAT CAA GCG GTA ACG AAC GCG AGC AGC CCT TCA GAA ATC AAT GCC ACG	477
Asn Gln Ala Val Thr Asn Ala Ser Ser Pro Ser Glu Ile Asn Ala Thr	
50 55 60	
ATC GAT AAT TTA AAA GCA AAC ACG CAA GGG CTG ATT GGC GAA AAA ACC	525
Ile Asp Asn Leu Lys Ala Asn Thr Gln Gly Leu Ile Gly Glu Lys Thr	
65 70 75	
AAT TCC CCG GCG TAT CAA GCG GTG TAT TTG GCG CTC AAT GCG GCG GTG	573
Asn Ser Pro Ala Tyr Gln Ala Val Tyr Leu Ala Leu Asn Ala Ala Val	
80 85 90	
GGG CTG TGG AAT GTG ATA GCC TAT AAT GTC CAA TGC GGT CCT GGT AAG	621
Gly Leu Trp Asn Val Ile Ala Tyr Asn Val Gln Cys Gly Pro Gly Lys	
95 100 105 110	
AGT GGG GAT CAA AGC GTA ATT TTT GAT GGC CAA CCA GGA CAT GAT TCA	669
Ser Gly Asp Gln Ser Val Ile Phe Asp Gly Gln Pro Gly His Asp Ser	
115 120 125	
AGA TCC ATT AAT TGC AAT TTA ACC GGT TAT AAC AAC GGG GTT AGC GGC	717
Arg Ser Ile Asn Cys Asn Leu Thr Gly Tyr Asn Asn Gly Val Ser Gly	
130 135 140	
CCT TTA TCC ATT GAC AAT TTT AAA ACG CTT AAT CAA GCT TAT CAA ACT	765
Pro Leu Ser Ile Asp Asn Phe Lys Thr Leu Asn Gln Ala Tyr Gln Thr	
145 150 155	
ATC CAA CAA GCT TTA AAA CAA GAT AGC GGA TTT CCT GTT TTG GAT AGT	813
Ile Gln Gln Ala Leu Lys Gln Asp Ser Gly Phe Pro Val Leu Asp Ser	
160 165 170	
AAA GGA AAA CAA GTA ACT ATA AAA ATA ACA ACA CAA ACT AAT GGA GCT	861
Lys Gly Lys Gln Val Thr Ile Lys Ile Thr Thr Gln Thr Asn Gly Ala	
175 180 185 190	
AAT AAA AGT GAA ACT ACT ACT ACT ACT ACT ACT ACT AAT GAC GCT CAA	909
Asn Lys Ser Glu Thr Thr Thr Thr Thr Thr Thr Thr Asn Asp Ala Gln	
195 200 205	
ACC CTT TTG CAA GAA GCC AGT AAA ATG ATA AGC GTC CTC ACT ACA AAC	957
Thr Leu Leu Gln Glu Ala Ser Lys Met Ile Ser Val Leu Thr Thr Asn	
210 215 220	
TGC CCA TGG GTA AAT ACC GCT CAT AAC TCA AAC GGG GGT GCA CCG TGG	1005
Cys Pro Trp Val Asn Thr Ala His Asn Ser Asn Gly Gly Ala Pro Trp	
225 230 235	
AAT TTA AAT ACG ACA GGG AAT GTG TGT CAG GTT TTT GCC ACG GAG TTT	1053
Asn Leu Asn Thr Thr Gly Asn Val Cys Gln Val Phe Ala Thr Glu Phe	
240 245 250	
AGC GCC GTT ACT AGC ATG ATC AAA AAC GCG CAA GAA ATC GTA ACG CAA	1101

Ser Ala Val Thr	Ser Met Ile Lys Asn Ala Gln Glu Ile Val Thr Gln	
255	260	265 270
GCT CAA AGC CTT AAC AAC CCG CAA AGC AAT CAA AAC GCG CCG AAA GAT		1149
Ala Gln Ser Leu Asn Asn Pro Gln Ser Asn Gln Asn Ala Pro Lys Asp		
275	280	285
TTC AAT CCT TAC ACC TCT GCT GAT AGG GCT TTC GCT CAA AAC ATG CTC		1197
Phe Asn Pro Tyr Thr Ser Ala Asp Arg Ala Phe Ala Gln Asn Met Leu		
290	295	300
AAT CAC GCG CAA GCG CAA GCC AAG ATG CTT GAA CTA GCC GAT CAA ATG		1245
Asn His Ala Gln Ala Gln Ala Lys Met Leu Glu Leu Ala Asp Gln Met		
305	310	315
AAA AAA GAC CTT AAC ACT ATC CCA AAA CAA TTT ATC ACA AAC TAC TTG		1293
Lys Lys Asp Leu Asn Thr Ile Pro Lys Gln Phe Ile Thr Asn Tyr Leu		
320	325	330
GCA GCT TGC CGC AAT GGG GGT GGG ACA TTA CCT GAT GCA GGG GTT ACT		1341
Ala Ala Cys Arg Asn Gly Gly Gly Thr Leu Pro Asp Ala Gly Val Thr		
335	340	345 350
TCT AAC ACT TGG GGG GCC GGT TGC GCC TAT GTG GAA GAG ACG ATA ACC		1389
Ser Asn Thr Trp Gly Ala Gly Cys Ala Tyr Val Glu Glu Thr Ile Thr		
355	360	365
GCC CTA AAT AAC AGC CTT GCG CAT TTT GGC ACT CAA GCC GAT CAA ATC		1437
Ala Leu Asn Asn Ser Leu Ala His Phe Gly Thr Gln Ala Asp Gln Ile		
370	375	380
AAG CAA TCT GAG TTG TTG GCG CGC ACG ATA CTT GAT TTT AGA GGC AGC		1485
Lys Gln Ser Glu Leu Leu Ala Arg Thr Ile Leu Asp Phe Arg Gly Ser		
385	390	395
CTT AAG GAT TTA AAC AAC ACT TAT AAC AGC ATC ACC ACG ACC GCT TCA		1533
Leu Lys Asp Leu Asn Asn Thr Tyr Asn Ser Ile Thr Thr Thr Ala Ser		
400	405	410
AAC ACG CCC AAT TCC CCA TTC CTT AAA AAT TTG ATA AGC CAA TCC ACT		1581
Asn Thr Pro Asn Ser Pro Phe Leu Lys Asn Leu Ile Ser Gln Ser Thr		
415	420	425 430
AAC CCT AAT AAC CCC GGG GGC TTA CAG GCC GTT TAT CAA GTC AAC CAA		1629
Asn Pro Asn Asn Pro Gly Gly Leu Gln Ala Val Tyr Gln Val Asn Gln		
435	440	445
AGC GCT TAT TCG CAA TTA TTA AGC GCC ACG CAA GAA TTA GGG CAT AAC		1677
Ser Ala Tyr Ser Gln Leu Leu Ser Ala Thr Gln Glu Leu Gly His Asn		
450	455	460
CCT TTC AGA CGC GTT GGC TTA ATC AGC TCT CAA ACC AAC AAC GGT GCG		1725
Pro Phe Arg Arg Val Gly Leu Ile Ser Ser Gln Thr Asn Asn Gly Ala		
465	470	475
ATG AAT GGG ATC GGC GTG CAA ATA GGG TAT AAA CAA TTT TTT GGT GAA		1773
Met Asn Gly Ile Gly Val Gln Ile Gly Tyr Lys Gln Phe Phe Gly Glu		

480	485	490	
AAA AGA AGA TGG GGG TTA AGG TAT TAT GGT TTT TTT GAT TAC AAC CAT			1821
Lys Arg Arg Trp Gly Leu Arg Tyr Tyr Gly Phe Phe Asp Tyr Asn His			
495	500	505	510
GCT TAT ATC AAA TCC AGC TTT TTC AAC TCC GCC TCT GAT GTG TTC ACT			1869
Ala Tyr Ile Lys Ser Ser Phe Phe Asn Ser Ala Ser Asp Val Phe Thr			
	515	520	525
TAT GGG GTA GGA ACA GAT GTC CTC TAT AAC TTT ATC AAC GAT AAA GCC			1917
Tyr Gly Val Gly Thr Asp Val Leu Tyr Asn Phe Ile Asn Asp Lys Ala			
	530	535	540
ACC AAA AAC AAT AAG ATT TCT TTT GGG GTG TTT GGG GGG ATT GCG TTA			1965
Thr Lys Asn Asn Lys Ile Ser Phe Gly Val Phe Gly Gly Ile Ala Leu			
	545	550	555
GCT GGC ACT TCG TGG CTT AAT TCT CAA TAC GTG AAT TTA GCG ACA TTC			2013
Ala Gly Thr Ser Trp Leu Asn Ser Gln Tyr Val Asn Leu Ala Thr Phe			
	560	565	570
AAT AAT TTT TAC AGC GCT AAA ATG AAT GTG GCG AAT TTC CAA TTC TTA			2061
Asn Asn Phe Tyr Ser Ala Lys Met Asn Val Ala Asn Phe Gln Phe Leu			
	575	580	585
TTC AAC TTG GGC TTG AGA ATG AAT CTC GCT AAA AAC AAA AAG AAA GCG			2109
Phe Asn Leu Gly Leu Arg Met Asn Leu Ala Lys Asn Lys Lys Lys Ala			
	595	600	605
AGC GAT CAT GTA GCT CAG CAT GGC GTG GAA CTA GGC GTG AAG ATC CCT			2157
Ser Asp His Val Ala Gln His Gly Val Glu Leu Gly Val Lys Ile Pro			
	610	615	620
ACG ATC AAC ACG AAT TAC TAT TCT TTG CTA GGC ACT CAA CTC CAA TAC			2205
Thr Ile Asn Thr Asn Tyr Tyr Ser Leu Leu Gly Thr Gln Leu Gln Tyr			
	625	630	635
CGC AGG CTT TAT AGC GTG TAT TTG AAT TAT GTG TTT GCT TAC TAATATCTG			2256
Arg Arg Leu Tyr Ser Val Tyr Leu Asn Tyr Val Phe Ala Tyr			
	640	645	650
TCTTTTTGTG AAACCTCCCTT TTAAAGGGAT TTTTTTTGAA GCCTTTCTTT TTTTAAACCC			2316
TCTTTTTTGG GGGTCAAGCG TAAAATTAC CCCTATCCCT TTAAGAAAAT AAAATAAAG			2376
AAAATGCGTT TTATAACAAA ATAAGATCTA AAACAATAAA ACAAAAACCC A			2427

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 672 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
 (B) LOCATION: 1...20
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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Met Lys Lys Thr Leu Leu Leu Ser Leu Ser Leu Ser Leu Ser Ser Ser
-20          -15          -10          -5
Leu Leu Asn Ala Glu Asp Asn Gly Phe Phe Ile Ser Ala Gly Tyr Gln
      1              5              10
Ile Gly Glu Ala Ala Gln Met Val Lys Asn Thr Gly Glu Leu Lys Lys
      15              20              25
Leu Ser Asp Thr Tyr Glu Asn Leu Ser Asn Leu Leu Thr Asn Phe Asn
      30              35              40
Asn Leu Asn Gln Ala Val Thr Asn Ala Ser Ser Pro Ser Glu Ile Asn
      45              50              55              60
Ala Thr Ile Asp Asn Leu Lys Ala Asn Thr Gln Gly Leu Ile Gly Glu
      65              70              75
Lys Thr Asn Ser Pro Ala Tyr Gln Ala Val Tyr Leu Ala Leu Asn Ala
      80              85              90
Ala Val Gly Leu Trp Asn Val Ile Ala Tyr Asn Val Gln Cys Gly Pro
      95              100              105
Gly Lys Ser Gly Asp Gln Ser Val Ile Phe Asp Gly Gln Pro Gly His
      110              115              120
Asp Ser Arg Ser Ile Asn Cys Asn Leu Thr Gly Tyr Asn Asn Gly Val
      125              130              135              140
Ser Gly Pro Leu Ser Ile Asp Asn Phe Lys Thr Leu Asn Gln Ala Tyr
      145              150              155
Gln Thr Ile Gln Gln Ala Leu Lys Gln Asp Ser Gly Phe Pro Val Leu
      160              165              170
Asp Ser Lys Gly Lys Gln Val Thr Ile Lys Ile Thr Thr Gln Thr Asn
      175              180              185
Gly Ala Asn Lys Ser Glu Thr Thr Thr Thr Thr Thr Thr Thr Asn Asp
      190              195              200
Ala Gln Thr Leu Leu Gln Glu Ala Ser Lys Met Ile Ser Val Leu Thr
      205              210              215              220
Thr Asn Cys Pro Trp Val Asn Thr Ala His Asn Ser Asn Gly Gly Ala
      225              230              235
Pro Trp Asn Leu Asn Thr Thr Gly Asn Val Cys Gln Val Phe Ala Thr
      240              245              250
Glu Phe Ser Ala Val Thr Ser Met Ile Lys Asn Ala Gln Glu Ile Val
      255              260              265
Thr Gln Ala Gln Ser Leu Asn Asn Pro Gln Ser Asn Gln Asn Ala Pro
      270              275              280
Lys Asp Phe Asn Pro Tyr Thr Ser Ala Asp Arg Ala Phe Ala Gln Asn
      285              290              295              300
Met Leu Asn His Ala Gln Ala Gln Ala Lys Met Leu Glu Leu Ala Asp
      305              310              315
Gln Met Lys Lys Asp Leu Asn Thr Ile Pro Lys Gln Phe Ile Thr Asn
      320              325              330
Tyr Leu Ala Ala Cys Arg Asn Gly Gly Gly Thr Leu Pro Asp Ala Gly
      335              340              345
Val Thr Ser Asn Thr Trp Gly Ala Gly Cys Ala Tyr Val Glu Glu Thr
      350              355              360
Ile Thr Ala Leu Asn Asn Ser Leu Ala His Phe Gly Thr Gln Ala Asp

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365          370          375          380
Gln Ile Lys Gln Ser Glu Leu Leu Ala Arg Thr Ile Leu Asp Phe Arg
          385          390          395
Gly Ser Leu Lys Asp Leu Asn Asn Thr Tyr Asn Ser Ile Thr Thr Thr
          400          405          410
Ala Ser Asn Thr Pro Asn Ser Pro Phe Leu Lys Asn Leu Ile Ser Gln
          415          420          425
Ser Thr Asn Pro Asn Asn Pro Gly Gly Leu Gln Ala Val Tyr Gln Val
          430          435          440
Asn Gln Ser Ala Tyr Ser Gln Leu Leu Ser Ala Thr Gln Glu Leu Gly
445          450          455          460
His Asn Pro Phe Arg Arg Val Gly Leu Ile Ser Ser Gln Thr Asn Asn
          465          470          475
Gly Ala Met Asn Gly Ile Gly Val Gln Ile Gly Tyr Lys Gln Phe Phe
          480          485          490
Gly Glu Lys Arg Arg Trp Gly Leu Arg Tyr Tyr Gly Phe Asp Tyr
          495          500          505
Asn His Ala Tyr Ile Lys Ser Ser Phe Phe Asn Ser Ala Ser Asp Val
          510          515          520
Phe Thr Tyr Gly Val Gly Thr Asp Val Leu Tyr Asn Phe Ile Asn Asp
525          530          535          540
Lys Ala Thr Lys Asn Asn Lys Ile Ser Phe Gly Val Phe Gly Gly Ile
          545          550          555
Ala Leu Ala Gly Thr Ser Trp Leu Asn Ser Gln Tyr Val Asn Leu Ala
          560          565          570
Thr Phe Asn Asn Phe Tyr Ser Ala Lys Met Asn Val Ala Asn Phe Gln
          575          580          585
Phe Leu Phe Asn Leu Gly Leu Arg Met Asn Leu Ala Lys Asn Lys Lys
          590          595          600
Lys Ala Ser Asp His Val Ala Gln His Gly Val Glu Leu Gly Val Lys
605          610          615          620
Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Leu Leu Gly Thr Gln Leu
          625          630          635
Gln Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn Tyr Val Phe Ala Tyr
          640          645          650

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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2429 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 205...2277
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 205...259
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGAAAGAAGA CTGATTAGTC TTTCTTTTAG GGGCGATTCA AGCCTTAAAA GCCGGGTCAA	60
AATCCCCATT TTTCCCAATT TTTACAAAAA AAAAAAAAAAC AAAATCTCTA AAATTTAGAG	120
CTAAAATTAG CCATAAAATT CCATTATTG CTTATAATAT GAAGTTTCTT TGTATCAAAG	180
AAAAATCTAT TAAAAGGAGA AAAC ATG AAA AAA TCC CTC TTA CTC TCT CTT	231
Met Lys Lys Ser Leu Leu Leu Ser Leu	
-15 -10	
TCT CTC ATC GCT TCC TTA TCA AGA GCT GAA GAT GAC GGA TTT TAT ACG	279
Ser Leu Ile Ala Ser Leu Ser Arg Ala Glu Asp Asp Gly Phe Tyr Thr	
-5 1 5	
AGT GTG GGC TAT CAG ATC GGT GAA GCG GTC CAA CAA GTG AAA AAC ACA	327
Ser Val Gly Tyr Gln Ile Gly Glu Ala Val Gln Gln Val Lys Asn Thr	
10 15 20	
GGA GCA TTG CAA AAT CTT GCA GAC AGA TAC GAT AAC TTA AAC AAC CTT	375
Gly Ala Leu Gln Asn Leu Ala Asp Arg Tyr Asp Asn Leu Asn Asn Leu	
25 30 35	
TTA AAC CAA TAC AAT TAT TTA AAT TCC TTA GTC AAT TTA GCC AGC ACG	423
Leu Asn Gln Tyr Asn Tyr Leu Asn Ser Leu Val Asn Leu Ala Ser Thr	
40 45 50 55	
CCG AGC GCG ATC ACC GGT GCG ATT GAT AAT TTA AGC TCA AGC GCG ATT	471
Pro Ser Ala Ile Thr Gly Ala Ile Asp Asn Leu Ser Ser Ser Ala Ile	
60 65 70	
AAC CTC ACT AGC GCC ACC ACC ACT TCC CCC GCC TAT CAA GCT GTG GCT	519
Asn Leu Thr Ser Ala Thr Thr Thr Ser Pro Ala Tyr Gln Ala Val Ala	
75 80 85	
TTA GCG CTC AAT GCC GCT GTG GGC ATG TGG CAA GTC ATA GCC CTT TTT	567
Leu Ala Leu Asn Ala Ala Val Gly Met Trp Gln Val Ile Ala Leu Phe	
90 95 100	
ATT GGC TGT GGC CCT GGC CCT ACC AAT AAT CAA AGC TAT CAA TCG TTT	615
Ile Gly Cys Gly Pro Gly Pro Thr Asn Asn Gln Ser Tyr Gln Ser Phe	
105 110 115	
GGT AAC ACA CCA GCC CTT AAT GGG ACC ACC ACC ACT TGC AAT CAA GCA	663
Gly Asn Thr Pro Ala Leu Asn Gly Thr Thr Thr Thr Cys Asn Gln Ala	
120 125 130 135	
TAT GGG ACA GGC CCT AAT GGC ATC CTA TCT ATT GAT GAA TAC CAA AAA	711
Tyr Gly Thr Gly Pro Asn Gly Ile Leu Ser Ile Asp Glu Tyr Gln Lys	
140 145 150	
CTC AAC CAA GCT TAT CAG ATC ATC CAA ACC GCT TTA AAC CAA AAT CAA	759
Leu Asn Gln Ala Tyr Gln Ile Ile Gln Thr Ala Leu Asn Gln Asn Gln	
155 160 165	
GGG GGT GGG ATG CCT GCC TTG AAT GAC ACC ACC AAA ACA GGG GTA GTC	807
Gly Gly Gly Met Pro Ala Leu Asn Asp Thr Thr Lys Thr Gly Val Val	

170	175	180	
AAC ATA CAA CAA ACC AAT TAT AGG ACC ACC ACA CAA AAC AAT ATC ATA Asn Ile Gln Gln Thr Asn Tyr Arg Thr Thr Thr Gln Asn Asn Ile Ile 185 190 195			855
GAG CAT TAT TAT ACA GAG AAT GGG AAA GAG ATC CCA GTC TCT TAT TCA Glu His Tyr Tyr Thr Glu Asn Gly Lys Glu Ile Pro Val Ser Tyr Ser 200 205 210 215			903
GGC GGA TCA TCA TTC TCG CCT ACA ATA CAA TTG ACA TAC CAT AAT AAC Gly Gly Ser Ser Phe Ser Pro Thr Ile Gln Leu Thr Tyr His Asn Asn 220 225 230			951
GCT GAA AAC CTT TTG CAA CAA GCC GCC ACT ATC ATG CAA GTC CTT ATT Ala Glu Asn Leu Leu Gln Gln Ala Ala Thr Ile Met Gln Val Leu Ile 235 240 245			999
ACT CAA AAG CCG CAT GTG CAA ACG AGC AAT GGC GGT AAA GCG TGG GGG Thr Gln Lys Pro His Val Gln Thr Ser Asn Gly Gly Lys Ala Trp Gly 250 255 260			1047
TTG AGT TCT ACG CCT GGG AAT GTG ATG GAT ATT TTT GGT CCT TCT TTT Leu Ser Ser Thr Pro Gly Asn Val Met Asp Ile Phe Gly Pro Ser Phe 265 270 275			1095
AAC GCT ATT AAT GAG ATG ATT AAA AAC GCT CAA ACA GCC CTA GCA AAA Asn Ala Ile Asn Glu Met Ile Lys Asn Ala Gln Thr Ala Leu Ala Lys 280 285 290 295			1143
ACC CAA CAG CTT AAC GCT AAT GAA AAC GCC CAA ATC ACG CAA CCC AAC Thr Gln Gln Leu Asn Ala Asn Glu Asn Ala Gln Ile Thr Gln Pro Asn 300 305 310			1191
AAT TTC AAC CCC TAC ACC TCT AAA GAC AAA GGG TTC GCT CAA GAA ATG Asn Phe Asn Pro Tyr Thr Ser Lys Asp Lys Gly Phe Ala Gln Glu Met 315 320 325			1239
CTC AAT AGA GCT GAA GCT CAA GCA GAG ATT TTA AAT TTA GCT AAG CAA Leu Asn Arg Ala Glu Ala Gln Ala Glu Ile Leu Asn Leu Ala Lys Gln 330 335 340			1287
GTA GCG AAC AAT TTC CAC AGC ATT CAA GGG CCT ATT CAA GGG GAT TTA Val Ala Asn Asn Phe His Ser Ile Gln Gly Pro Ile Gln Gly Asp Leu 345 350 355			1335
GAA GAA TGT AAA GCA GGA TCG GCT GGC GTG ATC ACT AAT AAC ACT TGG Glu Glu Cys Lys Ala Gly Ser Ala Gly Val Ile Thr Asn Asn Thr Trp 360 365 370 375			1383
GGT TCA GGT TGC GCG TTT GTG AAA GAA ACT TTA AAC TCT TTA GAG CAA Gly Ser Gly Cys Ala Phe Val Lys Glu Thr Leu Asn Ser Leu Glu Gln 380 385 390			1431
CAC ACC GCT TAT TAC GGC AAC CAG GTC AAT CAG GAT AGG GCT TTG GCT His Thr Ala Tyr Tyr Gly Asn Gln Val Asn Gln Asp Arg Ala Leu Ala 395 400 405			1479

CAA ACC ATT TTG AAT TTT AAA GAA GCC CTT AAC ACC CTG AAT AAA GAC	1527
Gln Thr Ile Leu Asn Phe Lys Glu Ala Leu Asn Thr Leu Asn Lys Asp	
410 415 420	
TCA AAA GCG ATC AAT AGC GGT ATC TCC AAC TTG CCT AAC GCT AAA TCT	1575
Ser Lys Ala Ile Asn Ser Gly Ile Ser Asn Leu Pro Asn Ala Lys Ser	
425 430 435	
CTT CAA AAC ATG ACG CAT GCC ACT CAA AAC CCT AAT TCC CCA GAA GGT	1623
Leu Gln Asn Met Thr His Ala Thr Gln Asn Pro Asn Ser Pro Glu Gly	
440 445 450 455	
CTG CTC ACT TAT TCT TTG GAT TCA AGC AAA TAC AAC CAG CTC CAA ACC	1671
Leu Leu Thr Tyr Ser Leu Asp Ser Ser Lys Tyr Asn Gln Leu Gln Thr	
460 465 470	
ATC GCG CAA GAA TTG GGC AAA AAC CCT TTC AGG CGC TTT GGC GTG ATT	1719
Ile Ala Gln Glu Leu Gly Lys Asn Pro Phe Arg Arg Phe Gly Val Ile	
475 480 485	
GAC TTT CAA AAC AAC AAC GGC GCA ATG AAC GGG ATC GGC GTG CAA GTG	1767
Asp Phe Gln Asn Asn Asn Gly Ala Met Asn Gly Ile Gly Val Gln Val	
490 495 500	
GGT TAT AAA CAA TTC TTT GGT AAA AAA AGG AAT TGG GGG TTA AGG TAT	1815
Gly Tyr Lys Gln Phe Phe Gly Lys Lys Arg Asn Trp Gly Leu Arg Tyr	
505 510 515	
TAT GGT TTC TTT GAT TAT AAC CAT GCT TAT ATC AAA TCT AAT TTT TTC	1863
Tyr Gly Phe Phe Asp Tyr Asn His Ala Tyr Ile Lys Ser Asn Phe Phe	
520 525 530 535	
AAC TCC GCT TCT GAT GTG TGG ACT TAT GGG GTG GGT ATG GAC GCT CTC	1911
Asn Ser Ala Ser Asp Val Trp Thr Tyr Gly Val Gly Met Asp Ala Leu	
540 545 550	
TAT AAC TTC ATC AAC GAT AAA AAC ACC AAC TTT TTA GGC AAG AAC AAC	1959
Tyr Asn Phe Ile Asn Asp Lys Asn Thr Asn Phe Leu Gly Lys Asn Asn	
555 560 565	
AAG CTT TCA GTA GGG CTT TTT GGA GGC TTT GCG TTA GCC GGG ACT TCG	2007
Lys Leu Ser Val Gly Leu Phe Gly Gly Phe Ala Leu Ala Gly Thr Ser	
570 575 580	
TGG CTT AAT TCC CAA CAA GTG AAT TTG ACC ATG ATG AAT GGC ATT TAT	2055
Trp Leu Asn Ser Gln Gln Val Asn Leu Thr Met Met Asn Gly Ile Tyr	
585 590 595	
AAC GCT AAT GTC AGC ACT TCT AAC TTC CAA TTT TTG TTT GAT TTA GGC	2103
Asn Ala Asn Val Ser Thr Ser Asn Phe Gln Phe Leu Phe Asp Leu Gly	
600 605 610 615	
TTG AGA ATG AAC CTC GCT AGG CCT AAG AAA AAA GAC AGC GAT CAT GCC	2151
Leu Arg Met Asn Leu Ala Arg Pro Lys Lys Lys Asp Ser Asp His Ala	
620 625 630	
GCT CAG CAT GGC ATT GAA CTA GGT TTT AAG ATC CCC ACG ATC AAC ACC	2199

Ala Gln His Gly Ile Glu Leu Gly Phe Lys Ile Pro Thr Ile Asn Thr
635 640 645

AAC TAT TAT TCT TTC ATG GGC GCT AAA CTA GAA TAC AGA AGG ATG TAT 2247
Asn Tyr Tyr Ser Phe Met Gly Ala Lys Leu Glu Tyr Arg Arg Met Tyr
650 655 660

AGC CTT TTT CTC AAT TAT GTG TTT GCT TAC TAAAAATTCT TTTTGAACCC CTC 2300
Ser Leu Phe Leu Asn Tyr Val Phe Ala Tyr
665 670

TTTTTTTGGG GGAGTGTTGC AAAAATGCCC CCCTATTTGC TTGTGAGTTT TGGTTAAAT 2360
TTTAGTTACC CACGCTTAAA AAGCGCCAAG CCTTTTACAC ACAACTCCTT TAATTTTGTT 2420
TTTAAGAAA 2429

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 691 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...18
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Lys Lys Ser Leu Leu Leu Ser Leu Ser Leu Ile Ala Ser Leu Ser
-15 -10 -5

Arg Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile Gly
1 5 10

Glu Ala Val Gln Gln Val Lys Asn Thr Gly Ala Leu Gln Asn Leu Ala
15 20 25 30

Asp Arg Tyr Asp Asn Leu Asn Asn Leu Leu Asn Gln Tyr Asn Tyr Leu
35 40 45

Asn Ser Leu Val Asn Leu Ala Ser Thr Pro Ser Ala Ile Thr Gly Ala
50 55 60

Ile Asp Asn Leu Ser Ser Ser Ala Ile Asn Leu Thr Ser Ala Thr Thr
65 70 75

Thr Ser Pro Ala Tyr Gln Ala Val Ala Leu Ala Leu Asn Ala Ala Val
80 85 90

Gly Met Trp Gln Val Ile Ala Leu Phe Ile Gly Cys Gly Pro Gly Pro
95 100 105 110

Thr Asn Asn Gln Ser Tyr Gln Ser Phe Gly Asn Thr Pro Ala Leu Asn
115 120 125

Gly Thr Thr Thr Thr Cys Asn Gln Ala Tyr Gly Thr Gly Pro Asn Gly
130 135 140

Ile Leu Ser Ile Asp Glu Tyr Gln Lys Leu Asn Gln Ala Tyr Gln Ile
145 150 155

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Ile Gln Thr Ala Leu Asn Gln Asn Gln Gly Gly Gly Met Pro Ala Leu
  160                      165                      170
Asn Asp Thr Thr Lys Thr Gly Val Val Asn Ile Gln Gln Thr Asn Tyr
  175                      180                      185                      190
Arg Thr Thr Thr Gln Asn Asn Ile Ile Glu His Tyr Tyr Thr Glu Asn
                      195                      200                      205
Gly Lys Glu Ile Pro Val Ser Tyr Ser Gly Gly Ser Ser Phe Ser Pro
                      210                      215                      220
Thr Ile Gln Leu Thr Tyr His Asn Asn Ala Glu Asn Leu Leu Gln Gln
                      225                      230                      235
Ala Ala Thr Ile Met Gln Val Leu Ile Thr Gln Lys Pro His Val Gln
                      240                      245                      250
Thr Ser Asn Gly Gly Lys Ala Trp Gly Leu Ser Ser Thr Pro Gly Asn
  255                      260                      265                      270
Val Met Asp Ile Phe Gly Pro Ser Phe Asn Ala Ile Asn Glu Met Ile
                      275                      280                      285
Lys Asn Ala Gln Thr Ala Leu Ala Lys Thr Gln Gln Leu Asn Ala Asn
                      290                      295                      300
Glu Asn Ala Gln Ile Thr Gln Pro Asn Asn Phe Asn Pro Tyr Thr Ser
                      305                      310                      315
Lys Asp Lys Gly Phe Ala Gln Glu Met Leu Asn Arg Ala Glu Ala Gln
                      320                      325                      330
Ala Glu Ile Leu Asn Leu Ala Lys Gln Val Ala Asn Asn Phe His Ser
  335                      340                      345                      350
Ile Gln Gly Pro Ile Gln Gly Asp Leu Glu Glu Cys Lys Ala Gly Ser
                      355                      360                      365
Ala Gly Val Ile Thr Asn Asn Thr Trp Gly Ser Gly Cys Ala Phe Val
                      370                      375                      380
Lys Glu Thr Leu Asn Ser Leu Glu Gln His Thr Ala Tyr Tyr Gly Asn
                      385                      390                      395
Gln Val Asn Gln Asp Arg Ala Leu Ala Gln Thr Ile Leu Asn Phe Lys
                      400                      405                      410
Glu Ala Leu Asn Thr Leu Asn Lys Asp Ser Lys Ala Ile Asn Ser Gly
  415                      420                      425                      430
Ile Ser Asn Leu Pro Asn Ala Lys Ser Leu Gln Asn Met Thr His Ala
                      435                      440                      445
Thr Gln Asn Pro Asn Ser Pro Glu Gly Leu Leu Thr Tyr Ser Leu Asp
                      450                      455                      460
Ser Ser Lys Tyr Asn Gln Leu Gln Thr Ile Ala Gln Glu Leu Gly Lys
                      465                      470                      475
Asn Pro Phe Arg Arg Phe Gly Val Ile Asp Phe Gln Asn Asn Asn Gly
                      480                      485                      490
Ala Met Asn Gly Ile Gly Val Gln Val Gly Tyr Lys Gln Phe Phe Gly
  495                      500                      505                      510
Lys Lys Arg Asn Trp Gly Leu Arg Tyr Tyr Gly Phe Phe Asp Tyr Asn
                      515                      520                      525
His Ala Tyr Ile Lys Ser Asn Phe Phe Asn Ser Ala Ser Asp Val Trp
                      530                      535                      540
Thr Tyr Gly Val Gly Met Asp Ala Leu Tyr Asn Phe Ile Asn Asp Lys
                      545                      550                      555
Asn Thr Asn Phe Leu Gly Lys Asn Asn Lys Leu Ser Val Gly Leu Phe
                      560                      565                      570
Gly Gly Phe Ala Leu Ala Gly Thr Ser Trp Leu Asn Ser Gln Gln Val
  575                      580                      585                      590
Asn Leu Thr Met Met Asn Gly Ile Tyr Asn Ala Asn Val Ser Thr Ser
                      595                      600                      605
Asn Phe Gln Phe Leu Phe Asp Leu Gly Leu Arg Met Asn Leu Ala Arg

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[illegible]

(2) INFORMATION FOR SEO ID NO:13:

(i) SEOUENCE CHARACTERISTICS:

- (A) LENGTH: 2270 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
(B) LOCATION: 130...2049
(D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
(B) LOCATION: 130...193
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATTGAGCGCA	TCAAAACACC	CTAAAACTTT	TTTGAAATCC	AATAAAATTTA	TGTTATAATT	60
AAACGCATTG	TAAATAAATT	CTCATTTTGA	TACATTTTTA	CAATAAAACA	TTACTTTAAG	120
GAACATCTT	ATG AAA AAA	ACG AAA AAA	ACG ATT CTG	CTT TCT CTA	ACT CTC	171
	Met Lys Lys Thr	Lys Lys Thr	Ile Leu Leu	Ser Leu Thr	Leu	
	-20		-15		-10	
GCG GCG TCA	TTG CTC CAT	GCT GAA GAC	AAC GGC GTT	TTT TTA AGC	GTG	219
Ala Ala Ser	Leu Leu His	Ala Ala Glu	Asp Asn Gly	Val Phe Leu	Ser Val	
	-5		1		5	
GGT TAT CAA	ATC GGT GAA	GCG GTT CAA	AAA GTG AAA	AAC GCC GAC	AAG	267
Gly Tyr Gln	Ile Gly Glu	Ala Val Gln	Lys Val Lys	Asn Ala Asp	Lys	
10		15		20	25	
GTG CAA AAA	CTT TCA GAC	ACT TAT GAA	CAA TTA AGC	CGG CTT TTA	ACC	315
Val Gln Lys	Leu Ser Asp	Thr Tyr Glu	Gln Leu Ser	Arg Leu Leu	Thr	
	30		35		40	
AAC GAT AAT	GGC ACA AAC	TCA AAG ACA	AGC GCG CAA	ATC AAC CAA	GCG	363
Asn Asp Asn	Gly Thr Asn	Ser Lys Thr	Ser Ala Gln	Ile Asn Gln	Ala	
	45		50		55	

GTT AAT AAT TTG AAC GAA CGC GCA AAA ACT TTA GCC GGT GGG ACA ACC	411
Val Asn Asn Leu Asn Glu Arg Ala Lys Thr Leu Ala Gly Gly Thr Thr	
60 65 70	
AAT TCC CCT GCC TAT CAA GCC ACG CTT TTA GCG TTG AGA TCG GTG TTA	459
Asn Ser Pro Ala Tyr Gln Ala Thr Leu Leu Ala Leu Arg Ser Val Leu	
75 80 85	
GGG CTA TGG AAT AGC ATG GGT TAT GCG GTC ATA TGC GGA GGT TAT ACC	507
Gly Leu Trp Asn Ser Met Gly Tyr Ala Val Ile Cys Gly Gly Tyr Thr	
90 95 100 105	
AAA AGT CCA GGC GAA AAC AAT CAA AAA GAT TTC CAC TAC ACC GAT GAG	555
Lys Ser Pro Gly Glu Asn Asn Gln Lys Asp Phe His Tyr Thr Asp Glu	
110 115 120	
AAT GGC AAT GGC ACT ACA ATC AAT TGC GGT GGG AGC ACA AAT AGT AAT	603
Asn Gly Asn Gly Thr Thr Ile Asn Cys Gly Gly Ser Thr Asn Ser Asn	
125 130 135	
GGC ACT CAT AGT TCT AGT GGC ACA AAT ACA TTA AAA GCA GAC AAA AAT	651
Gly Thr His Ser Ser Ser Gly Thr Asn Thr Leu Lys Ala Asp Lys Asn	
140 145 150	
GTT TCT CTA TCT ATT GAG CAA TAT GAA AAA ATC CAT GAA GCT TAT CAG	699
Val Ser Leu Ser Ile Glu Gln Tyr Glu Lys Ile His Glu Ala Tyr Gln	
155 160 165	
ATT CTT TCA AAA GCT TTA AAA CAA GCC GGG CTT GCT CCT TTA AAT AGC	747
Ile Leu Ser Lys Ala Leu Lys Gln Ala Gly Leu Ala Pro Leu Asn Ser	
170 175 180 185	
AAA GGG GAA AAG TTA GAA GCG CAT GTA ACC ACA TCA AAA CCA GAA AAT	795
Lys Gly Glu Lys Leu Glu Ala His Val Thr Thr Ser Lys Pro Glu Asn	
190 195 200	
AAT AGT CAA ACT AAA ACG ACA ACT TCT GTT ATT GAT ACG ACT AAT GAT	843
Asn Ser Gln Thr Lys Thr Thr Thr Ser Val Ile Asp Thr Thr Asn Asp	
205 210 215	
GCG CAA AAT CTT TTG ACT CAA GCG CAA ACG ATT GTC AAT ACC CTT AAA	891
Ala Gln Asn Leu Leu Thr Gln Ala Gln Thr Ile Val Asn Thr Leu Lys	
220 225 230	
GAT TAT TGC CCC ATG TTG ATA GCG AAA TCT AGT AGT GAA AGT AGT GGC	939
Asp Tyr Cys Pro Met Leu Ile Ala Lys Ser Ser Ser Glu Ser Ser Gly	
235 240 245	
GCA GCT ACT ACA AAC GCC CCT TCA TGG CAA ACA GCC GGT GGC GGC AAA	987
Ala Ala Thr Thr Asn Ala Pro Ser Trp Gln Thr Ala Gly Gly Gly Lys	
250 255 260 265	
AAT TCA TGT GCG ACT TTT GGT GCG GAG TTT AGT GCC GCT TCA GAC ATG	1035
Asn Ser Cys Ala Thr Phe Gly Ala Glu Phe Ser Ala Ala Ser Asp Met	
270 275 280	
ATT AAT AAT GCG CAA AAA ATC GTT CAA GAA ACC CAA CAA CTC AGC GCC	1083

Ile Asn Asn	Ala Gln Lys	Ile Val Gln	Glu Thr Gln	Gln Leu Ser	Ala	
285		290		295		
AAC CAA CCA	AAA AAT ATC	ACA CAA CCC	CAT AAT CTC	AAC CTT AAC	ACC	1131
Asn Gln Pro	Lys Asn Ile	Thr Gln Pro	His Asn Leu	Asn Leu Asn	Thr	
300		305		310		
CCT AGC AGT	CTT ACG GCT	TTA GCT CAA	AAA ATG CTC	AAA AAT GCG	CAA	1179
Pro Ser Ser	Leu Thr Ala	Leu Ala Gln	Lys Met Leu	Lys Asn Ala	Gln	
315		320		325		
TCT CAA GCA	GAA ATT TTA	AAA CTA GCC	AAT CAA GTG	GAG AGC GAT	TTT	1227
Ser Gln Ala	Glu Ile Leu	Lys Leu Ala	Asn Gln Val	Glu Ser Asp	Phe	
330		335		340	345	
AAC AAA CTT	TCT TCA GGC	CAT CTT AAA	GAC TAC ATA	GGG AAA TGC	GAT	1275
Asn Lys Leu	Ser Ser Gly	His Leu Lys	Asp Tyr Ile	Gly Lys Cys	Asp	
	350		355		360	
GCG AGC GCT	ATA AGC AGT	GCG AAT ATG	ACA ATG CAA	AAT CAA AAG	AAC	1323
Ala Ser Ala	Ile Ser Ser	Ala Asn Met	Thr Met Gln	Asn Gln Lys	Asn	
	365		370		375	
AAT TGG GGG	AAC GGG TGT	GCT GGC GTG	GAA GAA ACT	CTG TCT TCA	TTA	1371
Asn Trp Gly	Asn Gly Cys	Ala Gly Val	Glu Glu Thr	Leu Ser Ser	Leu	
	380		385		390	
AAA ACA AGT	GCC GCT GAT	TTT AAC AAC	CAA ACG CCA	CAA ATC AAT	CAA	1419
Lys Thr Ser	Ala Ala Asp	Phe Asn Asn	Gln Thr Pro	Gln Ile Asn	Gln	
	395		400		405	
GCG CAA AAC	CTA GCC AAC	ACC CTT ATT	CAA GAA CTT	GGC AAC AAC	CCT	1467
Ala Gln Asn	Leu Ala Asn	Thr Leu Ile	Gln Glu Leu	Gly Asn Asn	Pro	
410		415		420	425	
TTT AGG AAT	ATG GGC ATG	ATC GCT TCT	TCA ACC ACG	AAT AAC GGC	GCC	1515
Phe Arg Asn	Met Gly Met	Ile Ala Ser	Ser Thr Thr	Asn Asn Gly	Ala	
	430		435		440	
TTG AAT GGC	CTT GGG GTG	CAA GTG GGT	TAT AAG CAA	TTT TTT GGG	GAA	1563
Leu Asn Gly	Leu Gly Val	Gln Val Gln	Tyr Lys Gln	Phe Phe Gly	Glu	
	445		450		455	
AAG AAA AGA	TGG GGG TTA	AGG TAT TAT	GGT TTC TTT	GAT TAC AAC	CAC	1611
Lys Lys Arg	Trp Gly Leu	Arg Tyr Tyr	Gly Phe Phe	Asp Tyr Asn	His	
	460		465		470	
GCC TAT ATC	AAA TCC AAT	TTC TTT AAC	TCG GCT TCT	GAT GTG TGG	ACT	1659
Ala Tyr Ile	Lys Ser Asn	Phe Phe Asn	Ser Ala Ser	Asp Val Trp	Thr	
	475		480		485	
TAT GGG GTG	GGC AGC GAT	TTA TTG TTT	AAT TTC ATC	AAT GAT AAA	AAC	1707
Tyr Gly Val	Gly Ser Asp	Leu Leu Phe	Asn Phe Ile	Asn Asp Lys	Asn	
490		495		500	505	
ACC AAC TTT	TTA GGC AAG	AAT AAC AAG	ATT TCA GTG	GGA TTT TTT	GGA	1755
Thr Asn Phe	Leu Gly Lys	Asn Asn Lys	Ile Ser Val	Gly Phe Phe	Gly	

510	515	520	
GGT ATC GCC TTA GCA GGG ACT TCA TGG CTT AAT TCT CAA TTC GTG AAT			1803
Gly Ile Ala Leu Ala Gly Thr Ser Trp Leu Asn Ser Gln Phe Val Asn			
525	530	535	
TTA AAA ACC ATC AGC AAT GTT TAT AGC GCT AAA GTG AAT ACG GCT AAC			1851
Leu Lys Thr Ile Ser Asn Val Tyr Ser Ala Lys Val Asn Thr Ala Asn			
540	545	550	
TTC CAA TTT TTA TTC AAT TTG GGC TTG AGA ACC AAT CTC GCT AGA CCT			1899
Phe Gln Phe Leu Phe Asn Leu Gly Leu Arg Thr Asn Leu Ala Arg Pro			
555	560	565	
AAG AAA AAA GAT AGT CAT CAT GCG GCT CAA CAT GGC ATG GAA TTG GGC			1947
Lys Lys Lys Asp Ser His His Ala Ala Gln His Gly Met Glu Leu Gly			
570	575	580	585
GTG AAA ATC CCT ACC ATT AAC ACG AAT TAT TAT TCT TTT CTA GAC ACT			1995
Val Lys Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Phe Leu Asp Thr			
590	595	600	
AAA CTA GAA TAT CGA AGG CTT TAT AGC GTG TAT CTC AAT TAT GTG TTT			2043
Lys Leu Glu Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn Tyr Val Phe			
605	610	615	
GCC TAT TAAAAACCCT CTTTTTAAAA AAGGGGGGGC TTAAAAAAC CTCTAAAGAT AA			2101
Ala Tyr			
AAATTTTCAA AAAACAATCA TTAACCCTA AAAAAGAAAT TTTAAGGTAT AATGCTTTCG			2161
CCATTTTAA TTTTCCATGG CAAACTCCTT TTTAGAATT ATCCCCATAA TCGCTCTTAT			2221
GGGGCGTTTG TTTTGCAACA ATCTTTTCGA AACTATCCAA CAAGCTTTA			2270

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 640 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...21
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Lys Lys Thr Lys Lys Thr Ile Leu Leu Ser Leu Thr Leu Ala Ala
 -20 -15 -10
 Ser Leu Leu His Ala Glu Asp Asn Gly Val Phe Leu Ser Val Gly Tyr

-5		1		5		10
Gln Ile Gly Glu Ala Val Gln Lys Val Lys Asn Ala Asp Lys Val Gln						
	15		20		25	
Lys Leu Ser Asp Thr Tyr Glu Gln Leu Ser Arg Leu Leu Thr Asn Asp						
	30		35		40	
Asn Gly Thr Asn Ser Lys Thr Ser Ala Gln Ile Asn Gln Ala Val Asn						
	45		50		55	
Asn Leu Asn Glu Arg Ala Lys Thr Leu Ala Gly Gly Thr Thr Asn Ser						
	60		65		70	75
Pro Ala Tyr Gln Ala Thr Leu Leu Ala Leu Arg Ser Val Leu Gly Leu						
		80		85		90
Trp Asn Ser Met Gly Tyr Ala Val Ile Cys Gly Gly Tyr Thr Lys Ser						
	95		100			105
Pro Gly Glu Asn Asn Gln Lys Asp Phe His Tyr Thr Asp Glu Asn Gly						
	110		115		120	
Asn Gly Thr Thr Ile Asn Cys Gly Gly Ser Thr Asn Ser Asn Gly Thr						
	125		130		135	
His Ser Ser Ser Gly Thr Asn Thr Leu Lys Ala Asp Lys Asn Val Ser						
	140		145		150	155
Leu Ser Ile Glu Gln Tyr Glu Lys Ile His Glu Ala Tyr Gln Ile Leu						
		160		165		170
Ser Lys Ala Leu Lys Gln Ala Gly Leu Ala Pro Leu Asn Ser Lys Gly						
		175		180		185
Glu Lys Leu Glu Ala His Val Thr Thr Ser Lys Pro Glu Asn Asn Ser						
	190		195		200	
Gln Thr Lys Thr Thr Thr Ser Val Ile Asp Thr Thr Asn Asp Ala Gln						
	205		210		215	
Asn Leu Leu Thr Gln Ala Gln Thr Ile Val Asn Thr Leu Lys Asp Tyr						
	220		225		230	235
Cys Pro Met Leu Ile Ala Lys Ser Ser Ser Glu Ser Ser Gly Ala Ala						
		240		245		250
Thr Thr Asn Ala Pro Ser Trp Gln Thr Ala Gly Gly Gly Lys Asn Ser						
		255		260		265
Cys Ala Thr Phe Gly Ala Glu Phe Ser Ala Ala Ser Asp Met Ile Asn						
		270		275		280
Asn Ala Gln Lys Ile Val Gln Glu Thr Gln Gln Leu Ser Ala Asn Gln						
	285		290		295	
Pro Lys Asn Ile Thr Gln Pro His Asn Leu Asn Leu Asn Thr Pro Ser						
	300		305		310	315
Ser Leu Thr Ala Leu Ala Gln Lys Met Leu Lys Asn Ala Gln Ser Gln						
		320		325		330
Ala Glu Ile Leu Lys Leu Ala Asn Gln Val Glu Ser Asp Phe Asn Lys						
		335		340		345
Leu Ser Ser Gly His Leu Lys Asp Tyr Ile Gly Lys Cys Asp Ala Ser						
		350		355		360
Ala Ile Ser Ser Ala Asn Met Thr Met Gln Asn Gln Lys Asn Asn Trp						
	365		370		375	
Gly Asn Gly Cys Ala Gly Val Glu Glu Thr Leu Ser Ser Leu Lys Thr						
	380		385		390	395
Ser Ala Ala Asp Phe Asn Asn Gln Thr Pro Gln Ile Asn Gln Ala Gln						
		400		405		410
Asn Leu Ala Asn Thr Leu Ile Gln Glu Leu Gly Asn Asn Pro Phe Arg						
		415		420		425
Asn Met Gly Met Ile Ala Ser Ser Thr Thr Asn Asn Gly Ala Leu Asn						
	430		435		440	
Gly Leu Gly Val Gln Val Gly Tyr Lys Gln Phe Phe Gly Glu Lys Lys						
	445		450		455	

Arg Trp Gly Leu Arg Tyr Tyr Gly Phe Phe Asp Tyr Asn His Ala Tyr
 460 465 470 475
 Ile Lys Ser Asn Phe Phe Asn Ser Ala Ser Asp Val Trp Thr Tyr Gly
 480 485 490
 Val Gly Ser Asp Leu Leu Phe Asn Phe Ile Asn Asp Lys Asn Thr Asn
 495 500 505
 Phe Leu Gly Lys Asn Asn Lys Ile Ser Val Gly Phe Phe Gly Gly Ile
 510 515 520
 Ala Leu Ala Gly Thr Ser Trp Leu Asn Ser Gln Phe Val Asn Leu Lys
 525 530 535
 Thr Ile Ser Asn Val Tyr Ser Ala Lys Val Asn Thr Ala Asn Phe Gln
 540 545 550 555
 Phe Leu Phe Asn Leu Gly Leu Arg Thr Asn Leu Ala Arg Pro Lys Lys
 560 565 570
 Lys Asp Ser His His Ala Ala Gln His Gly Met Glu Leu Gly Val Lys
 575 580 585
 Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Phe Leu Asp Thr Lys Leu
 590 595 600
 Glu Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn Tyr Val Phe Ala Tyr
 605 610 615

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2248 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 173...2128
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 173...224
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGGTTTTATC GTTACAAAAT TCAACATTTC AAAGATAAAT AAGTTAAAAT ACCCCAAAAT 60
 CTTTTTTTTT TTTTGAAT CCAATCAATT TATAGTAAAA TTAGGTTTCAT TGTAATATA 120
 TTATCACTTC ATGATATTCT TACAACAAAA ACATTACTTT AAGGAACATT TT ATG AAA 178
 Met Lys

 AAG ACA ATT CTG CTC TCT CTC TCT GCT TCA TCG CTC TTG CAC GCT GAA 226
 Lys Thr Ile Leu Leu Ser Leu Ser Ala Ser Ser Leu Leu His Ala Glu
 -15 -10 -5 1
 GAC AAC GGC TTT TTT GTG AGC GCC GGC TAT CAA ATC GGC GAA GCG GTG 274

Asp Asn Gly Phe Phe Val Ser Ala Gly Tyr Gln Ile Gly Glu Ala Val	
5 10 15	
CAA ATG GTC AAA AAC ACC GGT GAA TTG AAA AAC TTG AAC GAA AAA TAC	322
Gln Met Val Lys Asn Thr Gly Glu Leu Lys Asn Leu Asn Glu Lys Tyr	
20 25 30	
GAG CAA TTA AGC CAG TAT TTA AAT CAA GTG GCT TCG TTG AAG CAA AGC	370
Glu Gln Leu Ser Gln Tyr Leu Asn Gln Val Ala Ser Leu Lys Gln Ser	
35 40 45	
ATT CAA AAC GCC AAC AAC ATT GAG CTG GTC AAT AGC TCT TTA AAC TAT	418
Ile Gln Asn Ala Asn Asn Ile Glu Leu Val Asn Ser Ser Leu Asn Tyr	
50 55 60 65	
TTA AAA AGC TTT ACC AAC AAC AAC TAT AAC AGC ACC ACC CAA TCG CCC	466
Leu Lys Ser Phe Thr Asn Asn Asn Tyr Asn Ser Thr Thr Gln Ser Pro	
70 75 80	
ATC TTT AAT GCC GTG CAA GCC GTT ATC ACT TCG GTA TTG GGT TTT TGG	514
Ile Phe Asn Ala Val Gln Ala Val Ile Thr Ser Val Leu Gly Phe Trp	
85 90 95	
AGT CTT TAT GCG GGG AAT TAC TTC ACT TTT TTT GTG GGT AAA AAG GTG	562
Ser Leu Tyr Ala Gly Asn Tyr Phe Thr Phe Phe Val Gly Lys Lys Val	
100 105 110	
GGT GAT AGT GGG CAA CCC GCT AGT GTC CAG GGT AAC CCT CCT TTT AAA	610
Gly Asp Ser Gly Gln Pro Ala Ser Val Gln Gly Asn Pro Pro Phe Lys	
115 120 125	
ACG ATT ATA GAG AAC TGC TCA GGA ATT GAA AAC TGC GCT ATG GAT CAA	658
Thr Ile Ile Glu Asn Cys Ser Gly Ile Glu Asn Cys Ala Met Asp Gln	
130 135 140 145	
ACC ACT TAT GAT AAG ATG AAA AAA CTC GCT GAA GAC CTC CAA GCG GCT	706
Thr Thr Tyr Asp Lys Met Lys Lys Leu Ala Glu Asp Leu Gln Ala Ala	
150 155 160	
CAA ACA AAC TCT GCC ACT AAA GGC AAC AAT CTT TGC GCT TTA TCC GGG	754
Gln Thr Asn Ser Ala Thr Lys Gly Asn Asn Leu Cys Ala Leu Ser Gly	
165 170 175	
TGT GCT GCA ACA GAC TCA ACA TCA AAC CCA CCA AAC TCA ACC GTG AGC	802
Cys Ala Ala Thr Asp Ser Thr Ser Asn Pro Pro Asn Ser Thr Val Ser	
180 185 190	
AAC GCT CTT AAT TTG GCG CAA CAG CTT ATG GAT TTA ATC GCA AAC ACT	850
Asn Ala Leu Asn Leu Ala Gln Gln Leu Met Asp Leu Ile Ala Asn Thr	
195 200 205	
AAA ACG GCT ATG ATG TGG AAA AAT ATC GTC ATC AGT GGC GTT TCA AAC	898
Lys Thr Ala Met Met Trp Lys Asn Ile Val Ile Ser Gly Val Ser Asn	
210 215 220 225	
ACA TCC GGT GCT ATC ACA TCC ACT AAT TAC CCA ACG CAA TAC GCG GTG	946
Thr Ser Gly Ala Ile Thr Ser Thr Asn Tyr Pro Thr Gln Tyr Ala Val	

230										235										240									
TTT	AAC	AAC	ATT	AAG	GCG	ATG	ATA	CCC	ATT	TTG	CAA	CAA	GCG	GTT	ACG														
Phe	Asn	Asn	Ile	Lys	Ala	Met	Ile	Pro	Ile	Leu	Gln	Gln	Ala	Val	Thr														994
			245					250					255																
CTT	TCT	CAA	AGC	AAC	CAC	ACC	CTA	TCT	GCT	AGC	TTG	CAA	GCT	CAA	GCC														1042
Leu	Ser	Gln	Ser	Asn	His	Thr	Leu	Ser	Ala	Ser	Leu	Gln	Ala	Gln	Ala														
			260				265					270																	
ACA	GGA	TCT	CAA	ACA	AAC	CCT	AAA	TTC	GCT	AAA	GAC	ATC	TAC	ACT	TTC														1090
Thr	Gly	Ser	Gln	Thr	Asn	Pro	Lys	Phe	Ala	Lys	Asp	Ile	Tyr	Thr	Phe														
			275			280					285																		
GCT	CAA	AAC	CAA	AAG	CAA	GTC	ATC	TCT	TAC	GCT	CAA	GAC	ATT	TTC	AAC														1138
Ala	Gln	Asn	Gln	Lys	Gln	Val	Ile	Ser	Tyr	Ala	Gln	Asp	Ile	Phe	Asn														
			290			295				300					305														
CTC	TTT	AAT	TCT	ATC	CCT	GCA	GAG	CAG	TAT	AAG	TAT	CTA	GAG	AAA	GCT														1186
Leu	Phe	Asn	Ser	Ile	Pro	Ala	Glu	Gln	Tyr	Lys	Tyr	Leu	Glu	Lys	Ala														
				310				315						320															
TAC	TTG	AAA	ATA	CCC	AAT	GCG	GGT	TCA	ACG	CCT	ACT	AAC	CCT	TAC	AGA														1234
Tyr	Leu	Lys	Ile	Pro	Asn	Ala	Gly	Ser	Thr	Pro	Thr	Asn	Pro	Tyr	Arg														
				325			330					335																	
CAA	GTG	GTG	AAT	TTA	AAC	CAA	GAA	GTT	CAG	ACG	ATT	AAA	AAC	AAT	GTG														1282
Gln	Val	Val	Asn	Leu	Asn	Gln	Glu	Val	Gln	Thr	Ile	Lys	Asn	Asn	Val														
			340			345						350																	
AGT	TAT	TAT	GGT	AAC	CGG	GTG	GAT	GCG	GCT	TTA	AGC	GTG	GCT	AGA	GAT														1330
Ser	Tyr	Tyr	Gly	Asn	Arg	Val	Asp	Ala	Ala	Leu	Ser	Val	Ala	Arg	Asp														
			355			360					365																		
GTT	TAT	AAC	CTA	AAA	TCC	AAT	CAA	GCA	GAA	ATC	GTA	ACC	GCC	TAT	AAC														1378
Val	Tyr	Asn	Leu	Lys	Ser	Asn	Gln	Ala	Glu	Ile	Val	Thr	Ala	Tyr	Asn														
			370			375				380				385															
GAC	GCT	AAG	ACT	TTG	AGC	GAA	GAG	ATT	TCT	AAA	CTC	CCG	CAC	AAT	CAA														1426
Asp	Ala	Lys	Thr	Leu	Ser	Glu	Glu	Ile	Ser	Lys	Leu	Pro	His	Asn	Gln														
				390				395					400																
GTC	AAT	ACA	AAA	GAC	ATT	GTT	ACA	CTA	CCT	TAC	GAT	AAA	AAC	GCC	CCA														1474
Val	Asn	Thr	Lys	Asp	Ile	Val	Thr	Leu	Pro	Tyr	Asp	Lys	Asn	Ala	Pro														
			405				410					415																	
GCA	GCA	GGC	CAA	TCC	AAC	TAC	CAA	ATC	AAC	CCA	GAG	CAG	CAA	TCC	AAT														1522
Ala	Ala	Gly	Gln	Ser	Asn	Tyr	Gln	Ile	Asn	Pro	Glu	Gln	Gln	Ser	Asn														
			420				425					430																	
CTT	AAC	CAA	GCT	TTA	GCA	GCG	ATG	AGC	AAT	AAC	CCC	TTT	AAA	AAA	GTG														1570
Leu	Asn	Gln	Ala	Leu	Ala	Ala	Met	Ser	Asn	Asn	Pro	Phe	Lys	Lys	Val														
			435				440					445																	
GGC	ATG	ATC	AGC	TCT	CAA	AAC	AAT	AAC	GGC	GCT	TTG	AAC	GGG	CTT	GGC														1618
Gly	Met	Ile	Ser	Ser	Gln	Asn	Asn	Asn	Gly	Ala	Leu	Asn	Gly	Leu	Gly														
			450			455				460				465															

GTG CAA GTG GGT TAT AAG CAA TTC TTT GGC GAA AGC AAA AGA TGG GGG	1666
Val Gln Val Gly Tyr Lys Gln Phe Phe Gly Glu Ser Lys Arg Trp Gly	
470 475 480	
TTA AGG TAT TAC GGA TTC TTT GAT TAC AAC CAC GGC TAC ATC AAA TCC	1714
Leu Arg Tyr Tyr Gly Phe Phe Asp Tyr Asn His Gly Tyr Ile Lys Ser	
485 490 495	
AGC TTC TTT AAC TCT TCT TCT GAT ATA TGG ACT TAT GGC GGT GGG AGC	1762
Ser Phe Phe Asn Ser Ser Ser Asp Ile Trp Thr Tyr Gly Gly Gly Ser	
500 505 510	
GAT TTG TTA GTG AAT ATT ATC AAC GAT AGC ATC ACA AGA AAG AAC AAC	1810
Asp Leu Leu Val Asn Ile Ile Asn Asp Ser Ile Thr Arg Lys Asn Asn	
515 520 525	
AAG CTC TCC GTG GGT CTT TTT GGA GGC ATC CAA CTA GCA GGG ACT ACA	1858
Lys Leu Ser Val Gly Leu Phe Gly Gly Ile Gln Leu Ala Gly Thr Thr	
530 535 540 545	
TGG CTT AAT TCT CAA TAC GTG AAT TTA ACC GCG TTC AAT AAC CCT TAC	1906
Trp Leu Asn Ser Gln Tyr Val Asn Leu Thr Ala Phe Asn Asn Pro Tyr	
550 555 560	
AGC GCG AAA GTC AAT GCT ACC AAT TTC CAA TTC TTG TTC AAT CTC GGC	1954
Ser Ala Lys Val Asn Ala Thr Asn Phe Gln Phe Leu Phe Asn Leu Gly	
565 570 575	
TTG AGG ACG AAT CTC GCT ACA GCT AGG AAA AAA GAC AGC GAA CAT TCC	2002
Leu Arg Thr Asn Leu Ala Thr Ala Arg Lys Lys Asp Ser Glu His Ser	
580 585 590	
GCG CAA CAT GGC ATT GAA TTG GGT ATT AAA ATC CCC ACC ATT ACC ACG	2050
Ala Gln His Gly Ile Glu Leu Gly Ile Lys Ile Pro Thr Ile Thr Thr	
595 600 605	
AAT TAC TAT TCT TTT CTA GGC ACT CAA TTG CAA TAC AGA AGG CTC TAT	2098
Asn Tyr Tyr Ser Phe Leu Gly Thr Gln Leu Gln Tyr Arg Arg Leu Tyr	
610 615 620 625	
AGC GTG TAT CTC AAT TAT GTG TTC GCT TAC TGAGTGATTC AAGCTCTCTT CTT	2151
Ser Val Tyr Leu Asn Tyr Val Phe Ala Tyr	
630 635	
TAAGGGGGTT TAGAAAAATC GCAACGCCAA GCTTTTTATC GTTGGTGATA AAATCTACAA	2211
AACTAACGGC GCGACAACAA ACCCTAACGC TACGCTC	2248

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 652 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
 (B) LOCATION: 1...17
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

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Met Lys Lys Thr Ile Leu Leu Ser Leu Ser Ala Ser Ser Leu Leu His
      -15                      -10                      -5
Ala Glu Asp Asn Gly Phe Phe Val Ser Ala Gly Tyr Gln Ile Gly Glu
   1          5          10          15
Ala Val Gln Met Val Lys Asn Thr Gly Glu Leu Lys Asn Leu Asn Glu
      20          25          30
Lys Tyr Glu Gln Leu Ser Gln Tyr Leu Asn Gln Val Ala Ser Leu Lys
      35          40          45
Gln Ser Ile Gln Asn Ala Asn Asn Ile Glu Leu Val Asn Ser Ser Leu
      50          55          60
Asn Tyr Leu Lys Ser Phe Thr Asn Asn Asn Tyr Asn Ser Thr Thr Gln
      65          70          75
Ser Pro Ile Phe Asn Ala Val Gln Ala Val Ile Thr Ser Val Leu Gly
      80          85          90          95
Phe Trp Ser Leu Tyr Ala Gly Asn Tyr Phe Thr Phe Phe Val Gly Lys
      100         105         110
Lys Val Gly Asp Ser Gly Gln Pro Ala Ser Val Gln Gly Asn Pro Pro
      115         120         125
Phe Lys Thr Ile Ile Glu Asn Cys Ser Gly Ile Glu Asn Cys Ala Met
      130         135         140
Asp Gln Thr Thr Tyr Asp Lys Met Lys Lys Leu Ala Glu Asp Leu Gln
      145         150         155
Ala Ala Gln Thr Asn Ser Ala Thr Lys Gly Asn Asn Leu Cys Ala Leu
      160         165         170         175
Ser Gly Cys Ala Ala Thr Asp Ser Thr Ser Asn Pro Pro Asn Ser Thr
      180         185         190
Val Ser Asn Ala Leu Asn Leu Ala Gln Gln Leu Met Asp Leu Ile Ala
      195         200         205
Asn Thr Lys Thr Ala Met Met Trp Lys Asn Ile Val Ile Ser Gly Val
      210         215         220
Ser Asn Thr Ser Gly Ala Ile Thr Ser Thr Asn Tyr Pro Thr Gln Tyr
      225         230         235
Ala Val Phe Asn Asn Ile Lys Ala Met Ile Pro Ile Leu Gln Gln Ala
      240         245         250         255
Val Thr Leu Ser Gln Ser Asn His Thr Leu Ser Ala Ser Leu Gln Ala
      260         265         270
Gln Ala Thr Gly Ser Gln Thr Asn Pro Lys Phe Ala Lys Asp Ile Tyr
      275         280         285
Thr Phe Ala Gln Asn Gln Lys Gln Val Ile Ser Tyr Ala Gln Asp Ile
      290         295         300
Phe Asn Leu Phe Asn Ser Ile Pro Ala Glu Gln Tyr Lys Tyr Leu Glu
      305         310         315
Lys Ala Tyr Leu Lys Ile Pro Asn Ala Gly Ser Thr Pro Thr Asn Pro
      320         325         330         335
Tyr Arg Gln Val Val Asn Leu Asn Gln Glu Val Gln Thr Ile Lys Asn
      340         345         350
Asn Val Ser Tyr Tyr Gly Asn Arg Val Asp Ala Ala Leu Ser Val Ala

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SUBSTITUTE SHEET (RULE 26)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CAAAAATCTT TTTT TTTT TTTTGAAATC CAATAAATTT ATGGTAAAGT TAAACATATT	60
GTAAATAAAT TTTAATTTCT ATTCATGTTT ACAATAAAAA AATTACTTTA AGGAACATTT	120
T ATG AAA AAG ACA ATT CTA CTC TCT CTC TCT CTC TCG CTT TCA TCG CTC	169
Met Lys Lys Thr Ile Leu Leu Ser Leu Ser Leu Ser Leu Ser Ser Leu	
-15 -10 -5	
TTG CAC GCT GAA GAC AAC GGC TTT TTT GTG AGC GCC GGC TAT CAA ATC	217
Leu His Ala Glu Asp Asn Gly Phe Phe Val Ser Ala Gly Tyr Gln Ile	
1 5 10	
GGC GAA CGG GTG CAA ATG GTC AAA AAC ACC GGC GAA TTG AAA AAC TTG	265
Gly Glu Arg Val Gln Met Val Lys Asn Thr Gly Glu Leu Lys Asn Leu	
15 20 25	
AAC GAA AAA TAC GAG CAA TTA AGC CAA TCT TTA GCC CAA CTG GCT TCG	313
Asn Glu Lys Tyr Glu Gln Leu Ser Gln Ser Leu Ala Gln Leu Ala Ser	
30 35 40 45	
TTA AAA AAA AGC ATT CAA ACG GCG AAC AAC ATT CAG GCT GTC AAC AAT	361
Leu Lys Lys Ser Ile Gln Thr Ala Asn Asn Ile Gln Ala Val Asn Asn	
50 55 60	
GCT TTA AGC GAT TTA AAA AGC TTT GCG AGT AAC AAC CAC ACA AAC AAA	409
Ala Leu Ser Asp Leu Lys Ser Phe Ala Ser Asn Asn His Thr Asn Lys	
65 70 75	
GAA ACA TCG CCC ATC TAC AAC ACC GCG CAA GCT GTT ATC ACT TCA GTA	457
Glu Thr Ser Pro Ile Tyr Asn Thr Ala Gln Ala Val Ile Thr Ser Val	
80 85 90	
TTG GCT TTT TGG AGT CTT TAT GCA GGG AAC GCT ACC AGT TTT CAT GTG	505
Leu Ala Phe Trp Ser Leu Tyr Ala Gly Asn Ala Thr Ser Phe His Val	
95 100 105	
ACC GGT TTG AAT GAT GGA TCT AAT GCT CCT CTT GGA AGA ATC CAT CAA	553
Thr Gly Leu Asn Asp Gly Ser Asn Ala Pro Leu Gly Arg Ile His Gln	
110 115 120 125	
GAT GGG AAC TGC ACA GGA TTA CAA CAA TGT TTT ATG AAT AAA GAA ACT	601
Asp Gly Asn Cys Thr Gly Leu Gln Gln Cys Phe Met Asn Lys Glu Thr	
130 135 140	
TAT GAT AAA ATG AAA GCG CTT GCC GAA AAT CTC CAA AAA GCT CAA GGC	649
Tyr Asp Lys Met Lys Ala Leu Ala Glu Asn Leu Gln Lys Ala Gln Gly	
145 150 155	
AAT CTC TGT GCC TTA TCA GAA TGC CCT AGC GAT CAA TTA AAT GGA AAC	697
Asn Leu Cys Ala Leu Ser Glu Cys Pro Ser Asp Gln Leu Asn Gly Asn	
160 165 170	
AAT GGA AAC AAA ACT TCC ATG ACT AAA GCT CTT GAA ACC GCG CAA CAG	745
Asn Gly Asn Lys Thr Ser Met Thr Lys Ala Leu Glu Thr Ala Gln Gln	
175 180 185	

CTT ATG GAT TTA ATC GCA AAC ACT AAA ACG GCT ATG ATG TGG AAA AAT	793
Leu Met Asp Leu Ile Ala Asn Thr Lys Thr Ala Met Met Trp Lys Asn	
190 195 200 205	
ATC GTC ATC GCA GGT GTT ACA AAC AGA CCC GGT GGT GCT GGC GCT ATC	841
Ile Val Ile Ala Gly Val Thr Asn Arg Pro Gly Gly Ala Gly Ala Ile	
210 215 220	
ACA TCC ACT GGT CCT GTA ACC GAC TAT GCG GTG TTT AAC AAC ATT AAG	889
Thr Ser Thr Gly Pro Val Thr Asp Tyr Ala Val Phe Asn Asn Ile Lys	
225 230 235	
GCG ATG ATA CCC ATT TTG CAA CAA GCG GTT ACG CTT TCT CAA AGC AAC	937
Ala Met Ile Pro Ile Leu Gln Gln Ala Val Thr Leu Ser Gln Ser Asn	
240 245 250	
CAC ACC CTA TCT GCT AGC TTG CAA GCT CAA GCC ACA GGA TCT CAA ACA	985
His Thr Leu Ser Ala Ser Leu Gln Ala Gln Ala Thr Gly Ser Gln Thr	
255 260 265	
AAC CCT AAA TTC GCT AAA GAC ATC TAC ACT TTC GCT CAA AAC CAA AAG	1033
Asn Pro Lys Phe Ala Lys Asp Ile Tyr Thr Phe Ala Gln Asn Gln Lys	
270 275 280 285	
CAA GTC ATC TCT TAC GCT CAA GAC ATT TTC AAC CTC TTT AAT TCT ATC	1081
Gln Val Ile Ser Tyr Ala Gln Asp Ile Phe Asn Leu Phe Asn Ser Ile	
290 295 300	
CCT GCA GAG CAG TAT AAG TAT CTA GAG AAA GCT TAC TTG AAA ATA CCC	1129
Pro Ala Glu Gln Tyr Lys Tyr Leu Glu Lys Ala Tyr Leu Lys Ile Pro	
305 310 315	
AAT GCG GGT TCA ACG CCT ACT AAC CCT TAC AGA CAA GTG GTG AAT TTA	1177
Asn Ala Gly Ser Thr Pro Thr Asn Pro Tyr Arg Gln Val Val Asn Leu	
320 325 330	
AAC CAA GAA GTT CAG ACG ATT AAA AAC AAT GTG AGT TAT TAT GGT AAC	1225
Asn Gln Glu Val Gln Thr Ile Lys Asn Asn Val Ser Tyr Tyr Gly Asn	
335 340 345	
CGG GTG GAT GCG GCT TTA AGC GTG GCT AGA GAT GTT TAT AAC CTA AAA	1273
Arg Val Asp Ala Ala Leu Ser Val Ala Arg Asp Val Tyr Asn Leu Lys	
350 355 360 365	
TCC AAT CAA GCA GAA ATC GTA ACC GCC TAT AAC GAC GCT AAG ACT TTG	1321
Ser Asn Gln Ala Glu Ile Val Thr Ala Tyr Asn Asp Ala Lys Thr Leu	
370 375 380	
AGC GAA GAG ATT TCT AAA CTC CCG CAC AAT CAA GTC AAT ACA AAA GAC	1369
Ser Glu Glu Ile Ser Lys Leu Pro His Asn Gln Val Asn Thr Lys Asp	
385 390 395	
ATT GTT ACA CTA CCT TAC GAT AAA AAC GCC CCA GCA GCA GGC CAA TCC	1417
Ile Val Thr Leu Pro Tyr Asp Lys Asn Ala Pro Ala Ala Gly Gln Ser	
400 405 410	
AAC TAC CAA ATC AAC CCA GAG CAG CAA TCC AAT CTT AAC CAA GCT TTA	1465

Asn Tyr Gln Ile Asn Pro Glu Gln Gln Ser Asn Leu Asn Gln Ala Leu	
415 420 425	
GCA GCG ATG AGC AAT AAC CCC TTT AAA AAA GTG GGC ATG ATC AGC TCT	1513
Ala Ala Met Ser Asn Asn Pro Phe Lys Lys Val Gly Met Ile Ser Ser	
430 435 440 445	
CAA AAC AAT AAC GGC GCT TTG AAC GGG CTT GGC GTG CAA GTG GGT TAT	1561
Gln Asn Asn Asn Gly Ala Leu Asn Gly Leu Gly Val Gln Val Gly Tyr	
450 455 460	
AAG CAA TTC TTT GGC GAA AGC AAA AGA TGG GGG TTA AGG TAT TAC GGA	1609
Lys Gln Phe Phe Gly Glu Ser Lys Arg Trp Gly Leu Arg Tyr Tyr Gly	
465 470 475	
TTC TTT GAT TAC AAC CAC GGC TAC ATC AAA TCC AGC TTC TTT AAC TCT	1657
Phe Phe Asp Tyr Asn His Gly Tyr Ile Lys Ser Ser Phe Phe Asn Ser	
480 485 490	
TCT TCT GAT ATA TGG ACT TAT GGC GGT GGG AGC GAT TTG TTA GTG AAT	1705
Ser Ser Asp Ile Trp Thr Tyr Gly Gly Gly Ser Asp Leu Leu Val Asn	
495 500 505	
ATT ATC AAC GAT AGC ATC ACA AGA AAG AAC AAC AAG CTC TCC GTG GGT	1753
Ile Ile Asn Asp Ser Ile Thr Arg Lys Asn Asn Lys Leu Ser Val Gly	
510 515 520 525	
CTT TTT GGA GGC ATC CAA CTA GCA GGG ACT ACA TGG CTT AAT TCT CAA	1801
Leu Phe Gly Gly Ile Gln Leu Ala Gly Thr Thr Trp Leu Asn Ser Gln	
530 535 540	
TAC GTG AAT TTA ACC GCG TTC AAT AAC CCT TAC AGC GCG AAA GTC AAT	1849
Tyr Val Asn Leu Thr Ala Phe Asn Asn Pro Tyr Ser Ala Lys Val Asn	
545 550 555	
GCT ACC AAT TTC CAA TTC TTG TTC AAT CTC GGC TTG AGG ACG AAT CTC	1897
Ala Thr Asn Phe Gln Phe Leu Phe Asn Leu Gly Leu Arg Thr Asn Leu	
560 565 570	
GCT ACA GCT AGG AAA AAA GAC AGC GAA CAT TCC GCG CAA CAT GGC ATT	1945
Ala Thr Ala Arg Lys Lys Asp Ser Glu His Ser Ala Gln His Gly Ile	
575 580 585	
GAA TTG GGT ATT AAA ATC CCC ACC ATT ACC ACG AAT TAC TAT TCT TTT	1993
Glu Leu Gly Ile Lys Ile Pro Thr Ile Thr Thr Asn Tyr Tyr Ser Phe	
590 595 600 605	
CTA GGC ACT CAA TTG CAA TAC AGA AGG CTC TAT AGC GTG TAT CTC AAT	2041
Leu Gly Thr Gln Leu Gln Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn	
610 615 620	
TAT GTG TTC GCT TAT TAAAAATCT TCTTTTAAA ATAGGGGGAG CTTTCATCAAA T	2097
Tyr Val Phe Ala Tyr	
625	
CTATTTTGAT AGTTATCAAT ATTTGATGAA AATAAAGTCA AAAACAAAAT AAACCAAATC	2157
ACCC	2161

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 645 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...19
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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Met Lys Lys Thr Ile Leu Leu Ser Leu Ser Leu Ser Leu Ser Ser Leu
      -15                      -10                      -5
Leu His Ala Glu Asp Asn Gly Phe Phe Val Ser Ala Gly Tyr Gln Ile
      1                      5                      10
Gly Glu Arg Val Gln Met Val Lys Asn Thr Gly Glu Leu Lys Asn Leu
      15                      20                      25
Asn Glu Lys Tyr Glu Gln Leu Ser Gln Ser Leu Ala Gln Leu Ala Ser
      30                      35                      40                      45
Leu Lys Lys Ser Ile Gln Thr Ala Asn Asn Ile Gln Ala Val Asn Asn
      50                      55                      60
Ala Leu Ser Asp Leu Lys Ser Phe Ala Ser Asn Asn His Thr Asn Lys
      65                      70                      75
Glu Thr Ser Pro Ile Tyr Asn Thr Ala Gln Ala Val Ile Thr Ser Val
      80                      85                      90
Leu Ala Phe Trp Ser Leu Tyr Ala Gly Asn Ala Thr Ser Phe His Val
      95                      100                      105
Thr Gly Leu Asn Asp Gly Ser Asn Ala Pro Leu Gly Arg Ile His Gln
      110                      115                      120                      125
Asp Gly Asn Cys Thr Gly Leu Gln Gln Cys Phe Met Asn Lys Glu Thr
      130                      135                      140
Tyr Asp Lys Met Lys Ala Leu Ala Glu Asn Leu Gln Lys Ala Gln Gly
      145                      150                      155
Asn Leu Cys Ala Leu Ser Glu Cys Pro Ser Asp Gln Leu Asn Gly Asn
      160                      165                      170
Asn Gly Asn Lys Thr Ser Met Thr Lys Ala Leu Glu Thr Ala Gln Gln
      175                      180                      185
Leu Met Asp Leu Ile Ala Asn Thr Lys Thr Ala Met Met Trp Lys Asn
      190                      195                      200                      205
Ile Val Ile Ala Gly Val Thr Asn Arg Pro Gly Gly Ala Gly Ala Ile
      210                      215                      220
Thr Ser Thr Gly Pro Val Thr Asp Tyr Ala Val Phe Asn Asn Ile Lys
      225                      230                      235
Ala Met Ile Pro Ile Leu Gln Gln Ala Val Thr Leu Ser Gln Ser Asn
      240                      245                      250
His Thr Leu Ser Ala Ser Leu Gln Ala Gln Ala Thr Gly Ser Gln Thr
      255                      260                      265
Asn Pro Lys Phe Ala Lys Asp Ile Tyr Thr Phe Ala Gln Asn Gln Lys

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270          275          280          285
Gln Val Ile Ser Tyr Ala Gln Asp Ile Phe Asn Leu Phe Asn Ser Ile
          290          295          300
Pro Ala Glu Gln Tyr Lys Tyr Leu Glu Lys Ala Tyr Leu Lys Ile Pro
          305          310          315
Asn Ala Gly Ser Thr Pro Thr Asn Pro Tyr Arg Gln Val Val Asn Leu
          320          325          330
Asn Gln Glu Val Gln Thr Ile Lys Asn Asn Val Ser Tyr Tyr Gly Asn
          335          340          345
Arg Val Asp Ala Ala Leu Ser Val Ala Arg Asp Val Tyr Asn Leu Lys
          350          355          360          365
Ser Asn Gln Ala Glu Ile Val Thr Ala Tyr Asn Asp Ala Lys Thr Leu
          370          375          380
Ser Glu Glu Ile Ser Lys Leu Pro His Asn Gln Val Asn Thr Lys Asp
          385          390          395
Ile Val Thr Leu Pro Tyr Asp Lys Asn Ala Pro Ala Ala Gly Gln Ser
          400          405          410
Asn Tyr Gln Ile Asn Pro Glu Gln Gln Ser Asn Leu Asn Gln Ala Leu
          415          420          425
Ala Ala Met Ser Asn Asn Pro Phe Lys Lys Val Gly Met Ile Ser Ser
          430          435          440          445
Gln Asn Asn Asn Gly Ala Leu Asn Gly Leu Gly Val Gln Val Gly Tyr
          450          455          460
Lys Gln Phe Phe Gly Glu Ser Lys Arg Trp Gly Leu Arg Tyr Tyr Gly
          465          470          475
Phe Phe Asp Tyr Asn His Gly Tyr Ile Lys Ser Ser Phe Phe Asn Ser
          480          485          490
Ser Ser Asp Ile Trp Thr Tyr Gly Gly Gly Ser Asp Leu Leu Val Asn
          495          500          505
Ile Ile Asn Asp Ser Ile Thr Arg Lys Asn Asn Lys Leu Ser Val Gly
          510          515          520          525
Leu Phe Gly Gly Ile Gln Leu Ala Gly Thr Thr Trp Leu Asn Ser Gln
          530          535          540
Tyr Val Asn Leu Thr Ala Phe Asn Asn Pro Tyr Ser Ala Lys Val Asn
          545          550          555
Ala Thr Asn Phe Gln Phe Leu Phe Asn Leu Gly Leu Arg Thr Asn Leu
          560          565          570
Ala Thr Ala Arg Lys Lys Asp Ser Glu His Ser Ala Gln His Gly Ile
          575          580          585
Glu Leu Gly Ile Lys Ile Pro Thr Ile Thr Thr Asn Tyr Tyr Ser Phe
          590          595          600          605
Leu Gly Thr Gln Leu Gln Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn
          610          615          620
Tyr Val Phe Ala Tyr
          625

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(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1799 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
 (B) LOCATION: 185...1633
 (D) OTHER INFORMATION:

(A) NAME/KEY: Signal Sequence
 (B) LOCATION: 185...233
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TACTCAAAAC ATTTTTCAC	ATCAAAAACC	TTTTTTTAA	ATCCAAAAAA	AAAGCAAAAT	60
TTCTTAATTT TTGCTCAATT	TTATTAAAAA	TTCAATAAAT	TTATGGCACA	ATTAAACTT	120
ATTGTAAATA AAGTTTCAAT	TTGATACGAT	TTTACAAACA	AAACATTACT	TTAAGGAACA	180
TTTT ATG AAA AAA ACG	ATT TTA CTT TCT CTT	ATG GTT TCA TCG	CTC CTC		229
Met Lys Lys Thr Ile Leu	Leu Ser Leu Met Val Ser	Ser Leu Leu			
-15	-10	-5			
GCT GAA AAT GAC GGC GTT	TTT ATG AGC GTG GGC	TAT CAA ATC GGC	GAA		277
Ala Glu Asn Asp Gly Val	Phe Met Ser Val Gly Tyr	Gln Ile Gly Glu			
1	5	10	15		
GCG GTT CAA CAA GTG	AAA AAC ACC GGC GAA	ATC CAA AAA GTC	TCC AAC		325
Ala Val Gln Gln Val Lys	Asn Thr Gly Glu Ile	Gln Lys Val Ser	Asn		
20	25	30			
GCT TAC GAA AAT TTG	AAC AAT CTT TTA ACC	CGC TAT AAC GAA	CTC AAA		373
Ala Tyr Glu Asn Leu Asn	Asn Leu Leu Thr Arg	Tyr Asn Glu Leu	Lys		
35	40	45			
CAA ACG GCC TCT AAC	ACC AAT TCA AGT ACC	GCT CAA GCG ATT	GAT AAT		421
Gln Thr Ala Ser Asn Thr	Asn Ser Ser Thr Ala	Gln Ala Ile Asp	Asn		
50	55	60			
CTA AAA GAG AGC GCT	AGC CGA TTG AAA ACG	ACC CCC AAT AGC	GCT AAT		469
Leu Lys Glu Ser Ala Ser	Arg Leu Lys Thr Thr	Pro Asn Ser Ala	Asn		
65	70	75			
CAA GCC GTG TCT TCA	GCG CTC AGC TCT GCG	GTA GCC ATG TGG	CAA GTA		517
Gln Ala Val Ser Ser Ala	Leu Ser Ser Ala Val	Ala Met Trp Gln	Val		
80	85	90	95		
ATA GTC TCT AAT TTA	GCC AAT AAC TCG CTA	CCC ACT AGT GAA	TAC AAC		565
Ile Val Ser Asn Leu Ala	Asn Asn Ser Leu Pro	Thr Ser Glu Tyr	Asn		
100	105	110			
AAA ATC AAT GCG ATT	TCT CAA TCG CTC CAA	AAC ACC CTA GAA	AAT AAA		613
Lys Ile Asn Ala Ile Ser	Gln Ser Leu Gln Asn	Thr Leu Glu Asn	Lys		
115	120	125			
AAC AAT GAT CTT AAA	ATT GAA AAT GAC TAC	GAC CAT CTT TTA	ACT CAA		661
Asn Asn Asp Leu Lys Ile	Glu Asn Asp Tyr Asp	His Leu Leu Thr	Gln		
130	135	140			

GCT AGC ACC ATT ATT AAT ACC CTT CAA AGC CAA TGC CCA GGC ATA GAC Ala Ser Thr Ile Ile Asn Thr Leu Gln Ser Gln Cys Pro Gly Ile Asp 145 150 155	709
GGA GGC AAT GGC AAA CCA TGG GGC ATT AAT GCA AGC GGG AAC GCA TGC Gly Gly Asn Gly Lys Pro Trp Gly Ile Asn Ala Ser Gly Asn Ala Cys 160 165 170 175	757
AAT ATT TTT GGC AAC ACC TTT AAC GCC ATC ACT AGC ATG ATA GAT AGC Asn Ile Phe Gly Asn Thr Phe Asn Ala Ile Thr Ser Met Ile Asp Ser 180 185 190	805
GCT AAA AAA GCC GCC GCA GAT GCC CGA AGA ACT GCC CCA GAA AGT CCA Ala Lys Lys Ala Ala Ala Asp Ala Arg Arg Thr Ala Pro Glu Ser Pro 195 200 205	853
AAC CAA CCA AGT GCG TTT AAC AAC GCT GAT TTC AAT AAA AAC CTT AAT Asn Gln Pro Ser Ala Phe Asn Asn Ala Asp Phe Asn Lys Asn Leu Asn 210 215 220	901
CAA GTC TCA AGC GTT ATT AAT GAC ACG ATC TCT TAC CTC AAA GGG GAC Gln Val Ser Ser Val Ile Asn Asp Thr Ile Ser Tyr Leu Lys Gly Asp 225 230 235	949
AAT TTA GCA ACC ATC TAC AAC ACC CTT CAA AAA ACG CCC GAT TCT AAA Asn Leu Ala Thr Ile Tyr Asn Thr Leu Gln Lys Thr Pro Asp Ser Lys 240 245 250 255	997
GGG TTT CAA AGT TTG GTG AGC CGA TCT AGC TAT AGT TAT TCC CTC AAC Gly Phe Gln Ser Leu Val Ser Arg Ser Ser Tyr Ser Tyr Ser Leu Asn 260 265 270	1045
GAA ACC CAA TAT TCT GAA TTC CAA ACT ACC ACC AAA GAG TTT GGC CAT Glu Thr Gln Tyr Ser Glu Phe Gln Thr Thr Thr Lys Glu Phe Gly His 275 280 285	1093
AAC CCT TTT AGA AGC GTG GGT TTA ATC AAC TCT CAA AGC AAT AAC GGA Asn Pro Phe Arg Ser Val Gly Leu Ile Asn Ser Gln Ser Asn Asn Gly 290 295 300	1141
GCG ATG AAT GGC GTG GGC GTG CAA TTA GGC TAT AAG CAA TTC TTT GGG Ala Met Asn Gly Val Gly Val Gln Leu Gly Tyr Lys Gln Phe Phe Gly 305 310 315	1189
AAA AAT AAA TTT TTT GGG ATC CGT TAT TAT GCC TTT TTT GAT TAC AAC Lys Asn Lys Phe Phe Gly Ile Arg Tyr Tyr Ala Phe Phe Asp Tyr Asn 320 325 330 335	1237
CAT GCC TAT ATC AAA TCC AAC TTT TTC AAC TCC GCT TCC AAT GTT TTC His Ala Tyr Ile Lys Ser Asn Phe Phe Asn Ser Ala Ser Asn Val Phe 340 345 350	1285
ACT TAT GGC GCA GGC AGT GAT CTT TTA TTG AAT TTC ATC AAT GGC GGA Thr Tyr Gly Ala Gly Ser Asp Leu Leu Leu Asn Phe Ile Asn Gly Gly 355 360 365	1333
TCC GAT AAA AAC CGC AAA GTC TCT TTT GGC ATT TTT GGA GGC ATC GCT	1381

Ser Asp Lys Asn Arg Lys Val Ser Phe Gly Ile Phe Gly Gly Ile Ala
 370 375 380

CTA GCA GGC ACG ACA TGG CTT AAT TCC CAA TTT ATG AAT TTA AAA ACC 1429
 Leu Ala Gly Thr Thr Trp Leu Asn Ser Gln Phe Met Asn Leu Lys Thr
 385 390 395

ACC AAT AGC GCC TAC AGC GCT AAG ATC AAC AAC ACC AAT TTC CAA TTC 1477
 Thr Asn Ser Ala Tyr Ser Ala Lys Ile Asn Asn Thr Asn Phe Gln Phe
 400 405 410 415

TTA TTC AAT ACT GGT TTA AGG CTT CAA GGG ATT CAC CAT GGC GTT GAA 1525
 Leu Phe Asn Thr Gly Leu Arg Leu Gln Gly Ile His His Gly Val Glu
 420 425 430

TTA GGC GTG AAA ATC CCC ACC ATC AAC ACG AAT TAC TAT TCT TTC ATG 1573
 Leu Gly Val Lys Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Phe Met
 435 440 445

GGC GCT AAA TTA GCA TAC CGA AGA CTT TAT AGC GTG TAT TTC AAT TAT 1621
 Gly Ala Lys Leu Ala Tyr Arg Arg Leu Tyr Ser Val Tyr Phe Asn Tyr
 450 455 460

GTT TTG GCC TAT TGATATTGAA TCGGTTCTCA TTACTAATGA GGACAAAGCC AACT 1678
 Val Leu Ala Tyr
 465

TTTTGGCTCT CAATGAATAA CGGCATCATT TTA CTGACT TTTTACAAAA AACACACTAA 1738
 AATTTCTTTT TCTTTTGTGA GCGAAATCC AGATTAGCTC AGCGGTAGAG TAGGCGGCTG 1798
 T 1799

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 483 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...16
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Lys Lys Thr Ile Leu Leu Ser Leu Met Val Ser Ser Leu Leu Ala
 -15 -10 -5

Glu Asn Asp Gly Val Phe Met Ser Val Gly Tyr Gln Ile Gly Glu Ala
 1 5 10 15

Val Gln Gln Val Lys Asn Thr Gly Glu Ile Gln Lys Val Ser Asn Ala
 20 25 30

Tyr Glu Asn Leu Asn Asn Leu Leu Thr Arg Tyr Asn Glu Leu Lys Gln
 35 40 45
 Thr Ala Ser Asn Thr Asn Ser Ser Thr Ala Gln Ala Ile Asp Asn Leu
 50 55 60
 Lys Glu Ser Ala Ser Arg Leu Lys Thr Thr Pro Asn Ser Ala Asn Gln
 65 70 75 80
 Ala Val Ser Ser Ala Leu Ser Ser Ala Val Ala Met Trp Gln Val Ile
 85 90 95
 Val Ser Asn Leu Ala Asn Asn Ser Leu Pro Thr Ser Glu Tyr Asn Lys
 100 105 110
 Ile Asn Ala Ile Ser Gln Ser Leu Gln Asn Thr Leu Glu Asn Lys Asn
 115 120 125
 Asn Asp Leu Lys Ile Glu Asn Asp Tyr Asp His Leu Leu Thr Gln Ala
 130 135 140
 Ser Thr Ile Ile Asn Thr Leu Gln Ser Gln Cys Pro Gly Ile Asp Gly
 145 150 155 160
 Gly Asn Gly Lys Pro Trp Gly Ile Asn Ala Ser Gly Asn Ala Cys Asn
 165 170 175
 Ile Phe Gly Asn Thr Phe Asn Ala Ile Thr Ser Met Ile Asp Ser Ala
 180 185 190
 Lys Lys Ala Ala Ala Asp Ala Arg Arg Thr Ala Pro Glu Ser Pro Asn
 195 200 205
 Gln Pro Ser Ala Phe Asn Asn Ala Asp Phe Asn Lys Asn Leu Asn Gln
 210 215 220
 Val Ser Ser Val Ile Asn Asp Thr Ile Ser Tyr Leu Lys Gly Asp Asn
 225 230 235 240
 Leu Ala Thr Ile Tyr Asn Thr Leu Gln Lys Thr Pro Asp Ser Lys Gly
 245 250 255
 Phe Gln Ser Leu Val Ser Arg Ser Ser Tyr Ser Tyr Ser Leu Asn Glu
 260 265 270
 Thr Gln Tyr Ser Glu Phe Gln Thr Thr Thr Lys Glu Phe Gly His Asn
 275 280 285
 Pro Phe Arg Ser Val Gly Leu Ile Asn Ser Gln Ser Asn Asn Gly Ala
 290 295 300
 Met Asn Gly Val Gly Val Gln Leu Gly Tyr Lys Gln Phe Phe Gly Lys
 305 310 315 320
 Asn Lys Phe Phe Gly Ile Arg Tyr Tyr Ala Phe Phe Asp Tyr Asn His
 325 330 335
 Ala Tyr Ile Lys Ser Asn Phe Phe Asn Ser Ala Ser Asn Val Phe Thr
 340 345 350
 Tyr Gly Ala Gly Ser Asp Leu Leu Asn Phe Ile Asn Gly Gly Ser
 355 360 365
 Asp Lys Asn Arg Lys Val Ser Phe Gly Ile Phe Gly Gly Ile Ala Leu
 370 375 380
 Ala Gly Thr Thr Trp Leu Asn Ser Gln Phe Met Asn Leu Lys Thr Thr
 385 390 395 400
 Asn Ser Ala Tyr Ser Ala Lys Ile Asn Asn Thr Asn Phe Gln Phe Leu
 405 410 415
 Phe Asn Thr Gly Leu Arg Leu Gln Gly Ile His His Gly Val Glu Leu
 420 425 430
 Gly Val Lys Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Phe Met Gly
 435 440 445
 Ala Lys Leu Ala Tyr Arg Arg Leu Tyr Ser Val Tyr Phe Asn Tyr Val
 450 455 460
 Leu Ala Tyr
 465

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2338 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 146...2218
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 146...200
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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ACTTAAAATT GTTTTTTTTT TTTTCAAAA TATAAATTTT AAGCCAAAAA TAAGCATTTT      60
ATGGTAAAAT GGCGAAC TTTT CATAAACATG ACTATTATGG GAATGTCATG GGAATGTGAA      120
GAAAAATCTA TAAAAA GGA GAA AAC ATG AAA AAA TCC CTC TTA CTC TCT CTT      172
                Met Lys Lys Ser Leu Leu Leu Ser Leu
                -18          -15          -10

TCT CTC ATC GCT TCC TTA TCA AGA GCT GAA GAT GAC GGA TTT TAT ACG      220
Ser Leu Ile Ala Ser Leu Ser Arg Ala Glu Asp Asp Gly Phe Tyr Thr
                -5          1          5

AGT GTG GGC TAT CAG ATC GGT GAA GCG GTC CAA CAA GTG AAA AAC ACA      268
Ser Val Gly Tyr Gln Ile Gly Glu Ala Val Gln Gln Val Lys Asn Thr
                10          15          20

GGA GCA TTG CAA AAT CTT GCA GAC AGA TAC GAT AAC TTA AAC AAC CTT      316
Gly Ala Leu Gln Asn Leu Ala Asp Arg Tyr Asp Asn Leu Asn Asn Leu
                25          30          35

TTA AAC CAA TAC AAT TAT TTA AAT TCC TTA GTC AAT TTA GCC AGC ACG      364
Leu Asn Gln Tyr Asn Tyr Leu Asn Ser Leu Val Asn Leu Ala Ser Thr
                40          45          50          55

CCG AGC GCG ATC ACC GGT GCG ATT GAT AAT TTA AGC TCA AGC GCG ATT      412
Pro Ser Ala Ile Thr Gly Ala Ile Asp Asn Leu Ser Ser Ser Ala Ile
                60          65          70

AAC CTC ACT AGC GCC ACC ACC ACT TCC CCC GCC TAT CAA GCT GTG GCT      460
Asn Leu Thr Ser Ala Thr Thr Thr Ser Pro Ala Tyr Gln Ala Val Ala
                75          80          85

TTA GCG CTC AAT GCC GCT GTG GGC ATG TGG CAA GTC ATA GCC CTT TTT      508
Leu Ala Leu Asn Ala Ala Val Gly Met Trp Gln Val Ile Ala Leu Phe

```

90	95	100	
ATT GGC TGT GGC CCT GGC CCT ACC AAT AAT CAA AGC TAT CAA TCG TTT Ile Gly Cys Gly Pro Gly Pro Thr Asn Asn Gln Ser Tyr Gln Ser Phe 105 110 115			556
GGT AAC ACA CCA GCC CTT AAT GGG ACC ACC ACC ACT TGC AAT CAA GCA Gly Asn Thr Pro Ala Leu Asn Gly Thr Thr Thr Thr Cys Asn Gln Ala 120 125 130 135			604
TAT GGG ACA GGC CCT AAT GGC ATC CTA TCT ATT GAT GAA TAC CAA AAA Tyr Gly Thr Gly Pro Asn Gly Ile Leu Ser Ile Asp Glu Tyr Gln Lys 140 145 150			652
CTC AAC CAA GCT TAT CAG ATC ATC CAA ACC GCT TTA AAC CAA AAT CAA Leu Asn Gln Ala Tyr Gln Ile Ile Gln Thr Ala Leu Asn Gln Asn Gln 155 160 165			700
GGG GGT GGG ATG CCT GCC TTG AAT GAC ACC ACC AAA ACA GGG GTA GTC Gly Gly Gly Met Pro Ala Leu Asn Asp Thr Thr Lys Thr Gly Val Val 170 175 180			748
AAC ATA CAA CAA ACC AAT TAT AGG ACC ACC ACA CAA AAC AAT ATC ATA Asn Ile Gln Gln Thr Asn Tyr Arg Thr Thr Thr Gln Asn Asn Ile Ile 185 190 195			796
GAG CAT TAT TAT ACA GAG AAT GGG AAA GAG ATC CCA GTC TCT TAT TCA Glu His Tyr Tyr Thr Glu Asn Gly Lys Glu Ile Pro Val Ser Tyr Ser 200 205 210 215			844
GGC GGA TCA TCA TTC TCG CCT ACA ATA CAA TTG ACA TAC CAT AAT AAC Gly Gly Ser Ser Phe Ser Pro Thr Ile Gln Leu Thr Tyr His Asn Asn 220 225 230			892
GCT GAA AAC CTT TTG CAA CAA GCC GCC ACT ATC ATG CAA GTC CTT ATT Ala Glu Asn Leu Leu Gln Gln Ala Ala Thr Ile Met Gln Val Leu Ile 235 240 245			940
ACT CAA AAG CCG CAT GTG CAA ACG AGC AAT GGC GGT AAA GCG TGG GGG Thr Gln Lys Pro His Val Gln Thr Ser Asn Gly Gly Lys Ala Trp Gly 250 255 260			988
TTG AGT TCT ACG CCT GGG AAT GTG ATG GAT ATT TTT GGT CCT TCT TTT Leu Ser Ser Thr Pro Gly Asn Val Met Asp Ile Phe Gly Pro Ser Phe 265 270 275			1036
AAC GCT ATT AAT GAG ATG ATT AAA AAC GCT CAA ACA GCC CTA GCA AAA Asn Ala Ile Asn Glu Met Ile Lys Asn Ala Gln Thr Ala Leu Ala Lys 280 285 290 295			1084
ACC CAA CAG CTT AAC GCT AAT GAA AAC GCC CAA ATC ACG CAA CCC AAC Thr Gln Gln Leu Asn Ala Asn Glu Asn Ala Gln Ile Thr Gln Pro Asn 300 305 310			1132
AAT TTC AAC CCC TAC ACC TCT AAA GAC AAA GGG TTC GCT CAA GAA ATG Asn Phe Asn Pro Tyr Thr Ser Lys Asp Lys Gly Phe Ala Gln Glu Met 315 320 325			1180

CTC AAT AGA GCT GAA GCT CAA GCA GAG ATT TTA AAT TTA GCT AAG CAA	1228
Leu Asn Arg Ala Glu Ala Gln Ala Glu Ile Leu Asn Leu Ala Lys Gln	
330 335 340	
GTA GCG AAC AAT TTC CAC AGC ATT CAA GGG CCT ATT CAA GGG GAT TTA	1276
Val Ala Asn Asn Phe His Ser Ile Gln Gly Pro Ile Gln Gly Asp Leu	
345 350 355	
GAA GAA TGT AAA GCA GGA TCG GCT GGC GTG ATC ACT AAT AAC ACT TGG	1324
Glu Glu Cys Lys Ala Gly Ser Ala Gly Val Ile Thr Asn Asn Thr Trp	
360 365 370 375	
GGT TCA GGT TGC GCG TTT GTG AAA GAA ACT TTA AAC TCT TTA GAG CAA	1372
Gly Ser Gly Cys Ala Phe Val Lys Glu Thr Leu Asn Ser Leu Glu Gln	
380 385 390	
CAC ACC GCT TAT TAC GGC AAC CAG GTC AAT CAG GAT AGG GCT TTG GCT	1420
His Thr Ala Tyr Tyr Gly Asn Gln Val Asn Gln Asp Arg Ala Leu Ala	
395 400 405	
CAA ACC ATT TTG AAT TTT AAA GAA GCC CTT AAC ACC CTG AAT AAA GAC	1468
Gln Thr Ile Leu Asn Phe Lys Glu Ala Leu Asn Thr Leu Asn Lys Asp	
410 415 420	
TCA AAA GCG ATC AAT AGC GGT ATC TCC AAC TTG CCT AAC GCT AAA TCT	1516
Ser Lys Ala Ile Asn Ser Gly Ile Ser Asn Leu Pro Asn Ala Lys Ser	
425 430 435	
CTT CAA AAC ATG ACG CAT GCC ACT CAA AAC CCT AAT TCC CCA GAA GGT	1564
Leu Gln Asn Met Thr His Ala Thr Gln Asn Pro Asn Ser Pro Glu Gly	
440 445 450 455	
CTG CTC ACT TAT TCT TTG GAT TCA AGC AAA TAC AAC CAG CTC CAA ACC	1612
Leu Leu Thr Tyr Ser Leu Asp Ser Ser Lys Tyr Asn Gln Leu Gln Thr	
460 465 470	
ATC GCG CAA GAA TTG GGC AAA AAC CCT TTC AGG CGC TTT GGC GTG ATT	1660
Ile Ala Gln Glu Leu Gly Lys Asn Pro Phe Arg Arg Phe Gly Val Ile	
475 480 485	
GAC TTT CAA AAC AAC AAC GGC GCA ATG AAC GGG ATC GGC GTG CAA GTG	1708
Asp Phe Gln Asn Asn Asn Gly Ala Met Asn Gly Ile Gly Val Gln Val	
490 495 500	
GGT TAT AAA CAA TTC TTT GGT AAA AAA AGG AAT TGG GGG TTA AGG TAT	1756
Gly Tyr Lys Gln Phe Phe Gly Lys Lys Arg Asn Trp Gly Leu Arg Tyr	
505 510 515	
TAT GGT TTC TTT GAT TAT AAC CAT GCT TAT ATC AAA TCT AAT TTT TTC	1804
Tyr Gly Phe Phe Asp Tyr Asn His Ala Tyr Ile Lys Ser Asn Phe Phe	
520 525 530 535	
AAC TCC GCT TCT GAT GTG TGG ACT TAT GGG GTG GGT ATG GAC GCT CTC	1852
Asn Ser Ala Ser Asp Val Trp Thr Tyr Gly Val Gly Met Asp Ala Leu	
540 545 550	
TAT AAC TTC ATC AAC GAT AAA AAC ACC AAC TTT TTA GGC AAG AAC AAC	1900

Tyr Asn Phe	Ile Asn Asp Lys Asn Thr Asn Phe Leu Gly	Lys Asn Asn	
555	560	565	
AAG CTT TCA GTA GGG CTT TTT GGA GGC TTT GCG TTA GCC GGG ACT TCG			1948
Lys Leu Ser Val Gly Leu Phe Gly Gly Phe Ala Leu Ala Gly Thr Ser			
570	575	580	
TGG CTT AAT TCC CAA CAA GTG AAT TTG ACC ATG ATG AAT GGC ATT TAT			1996
Trp Leu Asn Ser Gln Gln Val Asn Leu Thr Met Met Asn Gly Ile Tyr			
585	590	595	
AAC GCT AAT GTC AGC ACT TCT AAC TTC CAA TTT TTG TTT GAT TTA GGC			2044
Asn Ala Asn Val Ser Thr Ser Asn Phe Gln Phe Leu Phe Asp Leu Gly			
600	605	610	615
TTG AGA ATG AAC CTC GCT AGG CCT AAG AAA AAA GAC AGC GAT CAT GCC			2092
Leu Arg Met Asn Leu Ala Arg Pro Lys Lys Lys Asp Ser Asp His Ala			
620	625	630	
GCT CAG CAT GGC ATT GAA CTA GGT TTT AAG ATC CCC ACG ATC AAC ACC			2140
Ala Gln His Gly Ile Glu Leu Gly Phe Lys Ile Pro Thr Ile Asn Thr			
635	640	645	
AAC TAT TAT TCT TTC ATG GGC GCT AAA CTA GAA TAC AGA AGG ATG TAT			2188
Asn Tyr Tyr Ser Phe Met Gly Ala Lys Leu Glu Tyr Arg Arg Met Tyr			
650	655	660	
AGC CTT TTT CTC AAT TAT GTG TTT GCT TAC TAAAACTCT CTTTAAAAAA GGG			2241
Ser Leu Phe Leu Asn Tyr Val Phe Ala Tyr			
665	670		
GTTTGTTTAA AAACGCTTAA AAGCATTTT AAAATTAAGC AGTAAAGAGC CTAGATAATC			2301
TCTTGCAACC GCTCTCAAGC GATAAAATTA AAGTGAT			2338

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 691 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...18
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Lys Lys Ser Leu Leu Ser Leu Ser Leu Ile Ala Ser Leu Ser	
-18 -15 -10 -5	
Arg Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile Gly	

1	5	10
Glu Ala Val Gln Gln Val Lys Asn Thr Gly Ala Leu Gln Asn Leu Ala		
15	20	25
Asp Arg Tyr Asp Asn Leu Asn Asn Leu Leu Asn Gln Tyr Asn Tyr Leu		30
35	40	45
Asn Ser Leu Val Asn Leu Ala Ser Thr Pro Ser Ala Ile Thr Gly Ala		
50	55	60
Ile Asp Asn Leu Ser Ser Ser Ala Ile Asn Leu Thr Ser Ala Thr Thr		
65	70	75
Thr Ser Pro Ala Tyr Gln Ala Val Ala Leu Ala Leu Asn Ala Ala Val		
80	85	90
Gly Met Trp Gln Val Ile Ala Leu Phe Ile Gly Cys Gly Pro Gly Pro		
95	100	105
Thr Asn Asn Gln Ser Tyr Gln Ser Phe Gly Asn Thr Pro Ala Leu Asn		110
115	120	125
Gly Thr Thr Thr Thr Cys Asn Gln Ala Tyr Gly Thr Gly Pro Asn Gly		
130	135	140
Ile Leu Ser Ile Asp Glu Tyr Gln Lys Leu Asn Gln Ala Tyr Gln Ile		
145	150	155
Ile Gln Thr Ala Leu Asn Gln Asn Gln Gly Gly Gly Met Pro Ala Leu		
160	165	170
Asn Asp Thr Thr Lys Thr Gly Val Val Asn Ile Gln Gln Thr Asn Tyr		
175	180	185
Arg Thr Thr Thr Gln Asn Asn Ile Ile Glu His Tyr Tyr Thr Glu Asn		190
195	200	205
Gly Lys Glu Ile Pro Val Ser Tyr Ser Gly Gly Ser Ser Phe Ser Pro		
210	215	220
Thr Ile Gln Leu Thr Tyr His Asn Asn Ala Glu Asn Leu Leu Gln Gln		
225	230	235
Ala Ala Thr Ile Met Gln Val Leu Ile Thr Gln Lys Pro His Val Gln		
240	245	250
Thr Ser Asn Gly Gly Lys Ala Trp Gly Leu Ser Ser Thr Pro Gly Asn		
255	260	265
Val Met Asp Ile Phe Gly Pro Ser Phe Asn Ala Ile Asn Glu Met Ile		270
275	280	285
Lys Asn Ala Gln Thr Ala Leu Ala Lys Thr Gln Gln Leu Asn Ala Asn		
290	295	300
Glu Asn Ala Gln Ile Thr Gln Pro Asn Asn Phe Asn Pro Tyr Thr Ser		
305	310	315
Lys Asp Lys Gly Phe Ala Gln Glu Met Leu Asn Arg Ala Glu Ala Gln		
320	325	330
Ala Glu Ile Leu Asn Leu Ala Lys Gln Val Ala Asn Asn Phe His Ser		
335	340	345
Ile Gln Gly Pro Ile Gln Gly Asp Leu Glu Glu Cys Lys Ala Gly Ser		
355	360	365
Ala Gly Val Ile Thr Asn Asn Thr Trp Gly Ser Gly Cys Ala Phe Val		
370	375	380
Lys Glu Thr Leu Asn Ser Leu Glu Gln His Thr Ala Tyr Tyr Gly Asn		
385	390	395
Gln Val Asn Gln Asp Arg Ala Leu Ala Gln Thr Ile Leu Asn Phe Lys		
400	405	410
Glu Ala Leu Asn Thr Leu Asn Lys Asp Ser Lys Ala Ile Asn Ser Gly		
415	420	425
Ile Ser Asn Leu Pro Asn Ala Lys Ser Leu Gln Asn Met Thr His Ala		430
435	440	445
Thr Gln Asn Pro Asn Ser Pro Glu Gly Leu Leu Thr Tyr Ser Leu Asp		

[illegible]

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEOUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCAAGGAGAA AACATGAAAA AAACCC

26

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEO ID NO:24:

GAAGACGACG GCTTTTACAC AAGCGT

26

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAAGCTTAGT AAGCGAACAC ATAA

24

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAGGAGAAAA AACATGAAAA AACACATCC

29

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GAAGACGACG GCTTTTACAC AAGCG

25

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AACATTAGTA AGCGAACACA TAGTTC

26

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AAGGAGAAAA AACATGAAAA AACACATCC

29

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAAGACGACG GCTTTTACAC AAGCGT

26

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAAAGCTTAG TAAGCGAACA CAT

23

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAGGAGAAAA CATGAAGAAA AAATTT

26

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GAAGACAACG GCTTTTTTGT GAGTG

25

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AGCTTTTAGT AAGCAAACAC ATAGT

25

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AAGGATATTT ATGAAAAAAA CCCTT

25

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GAAGACAACG GCTTTTTTAT CAGCG

25

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GATATTAGTA AGCAAACACA TAATTC

26

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AAGGAGAAAA CATGAAAAAA TCCCTCT

27

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAAGATGACG GATTTTATAC GAGTGT

26

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TTTTAGTAAG CAAACACATA ATTGAG

26

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AAGGAACATC TTATGAAAAA AACG

24

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GAAGACAACG GCGTTTTTTT AAGCG

25

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GGTTTTTAAT AGGCAAACAC ATAAT

25

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AAGGAACATT TTATGAAAAA GACAAT

26

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GAAGACAACG GCTTTTTTGT GAGCG

25

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TCACTCAGTA AGCGAACACA TAA

23

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

AAGGAACATT TTATGAAAAA GACAA

25

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GAAGACAACG GCTTTTTTGT GAGCG

25

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TTTTAATAAG CGAACACATA AAAGAG

26

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

AAGGAACATT TTATGAAAAA AACGAT

26

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GAAAATGACG GCGTTTTTAT GAGCG

25

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ATATCAATAG GCCAAAACAT AATTGA

26

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AAGGAGAAAA CATGAAAAAA TCCCTC

26

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GAAGATGACG GATTTTATAC GAGTGT

26

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

TTTGTAGTAAG CAAACACATA ATTGAG

26

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CGCGGATCCG AATCCAATTT AATCCAAAAA GG

32

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

CCGCTCGAGT TAAGTAAGCG AACACATATT CAA

33

(2) INFORMATION FOR SEQ ID NO:58

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58

Glu	Asp	Asp	Gly	Phe	Tyr	Thr	Ser	Val	Gly	Tyr	Gln	Ile	Gly	Glu	Ala
1				5					10					15	
Ala	Gln	Met	Val												
			20												

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CTGAATTCGA TTTCAAGGAG AAAACATGAA A

31

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCGCTCGAGT TAGTAAGCGA ACACATAATT

30

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CGCGGATCCG AATCCAATTT AATCCAAAAA GG

32

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

CCGCTCGAGT TAGTAAGCGA ACACATAGTT CAA

33

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

CGCGGATCCG AAGTTTCTTT GTATCAAAG

29

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CCGCTCGAGT TAGTAAGCAA ACACATAATT GTG

33

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1149 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 106...1002

(D) OTHER INFORMATION:

(A) NAME/KEY: Signal Sequence

(B) LOCATION: 106...166

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

```

TTACTCTTTA ATGTGAGTTT TCTGTGTCAT GATAGCTGAT TTTGTTTAA ATTTGCTATA      60
ATGTGAATTT AATGATGAAA ATTAGTTTAG AGTGGAGAAC ACACA ATG AAA AAA AAT      117
                                         Met Lys Lys Asn
                                         -20
ATC TTA AAT TTA GCG TTA GTG GGT GCG TTG AGC ACG TCG TTT TTG ATG      165
Ile Leu Asn Leu Ala Leu Val Gly Ala Leu Ser Thr Ser Phe Leu Met
-15                               -10                               -5
GCT AAG CCG GCT CAT AAC GCA AAT AAC GCT ACG CAT AAC ACG AAA AAA      213
Ala Lys Pro Ala His Asn Ala Asn Asn Ala Thr His Asn Thr Lys Lys
1                               5                               10                               15
ACG ACT GAT TCT TCA GCA GGC GTG TTA GCG ACA GTG GAT GGC AGA CCT      261
Thr Thr Asp Ser Ser Ala Gly Val Leu Ala Thr Val Asp Gly Arg Pro
20                               25                               30
ATC ACT AAA AGC GAT TTT GAC ATG ATT AAG CAA CGA AAT CCT AAT TTT      309
Ile Thr Lys Ser Asp Phe Asp Met Ile Lys Gln Arg Asn Pro Asn Phe
35                               40                               45
GAT TTT GAC AAG CTT AAA GAG AAA GAA AAA GAA GCC TTG ATT GAT CAA      357
Asp Phe Asp Lys Leu Lys Glu Lys Glu Lys Glu Ala Leu Ile Asp Gln
50                               55                               60
GCT ATT CGC ACC GCC CTT GTA GAA AAT GAA GCT AAA ACC GAG AAA TTG      405
Ala Ile Arg Thr Ala Leu Val Glu Asn Glu Ala Lys Thr Glu Lys Leu
65                               70                               75                               80
GAC AGC ACT CCA GAA TTT AAA GCG ATG ATG GAA GCG GTT AAA AAA CAG      453
Asp Ser Thr Pro Glu Phe Lys Ala Met Met Glu Ala Val Lys Lys Gln
85                               90                               95
GCT TTA GTG GAA TTT TGG GCT AAA AAA CAG GCT GAA GAA GTG AAA AAA      501
Ala Leu Val Glu Phe Trp Ala Lys Lys Gln Ala Glu Glu Val Lys Lys
100                               105                               110
GTC CAA ATC CCA GAA AAA GAA ATG CAA GAT TTT TAC AAC GCT AAC AAA      549
Val Gln Ile Pro Glu Lys Glu Met Gln Asp Phe Tyr Asn Ala Asn Lys
115                               120                               125
GAT CAG CTT TTT GTC AAG CAA GAA GCC CAT GCT AGG CAT ATT TTA GTG      597
Asp Gln Leu Phe Val Lys Gln Glu Ala His Ala Arg His Ile Leu Val
130                               135                               140
AAA ACC GAA GAT GAG GCT AAA CGG ATT ATT TCT GAG ATT GAC AAA CAG      645
Lys Thr Glu Asp Glu Ala Lys Arg Ile Ile Ser Glu Ile Asp Lys Gln
145                               150                               155                               160
CCA AAG GCT AAA AAA GAA GCT AAA TTC ATT GAG TTA GCC AAT CGG GAT      693
Pro Lys Ala Lys Lys Glu Ala Lys Phe Ile Glu Leu Ala Asn Arg Asp
165                               170                               175

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ACG ATT GAT CCT AAC AGC AAG AAC GCG CAA AAT GGC GGT GAT TTG GGG      741
Thr Ile Asp Pro Asn Ser Lys Asn Ala Gln Asn Gly Gly Asp Leu Gly
      180      185      190
AAA TTC CAA AAG AAC CAA ATG GCT CCG GAT TTT TCT AAA GCC GCT TTC      789
Lys Phe Gln Lys Asn Gln Met Ala Pro Asp Phe Ser Lys Ala Ala Phe
      195      200      205
GCT TTA ACT CCT GGG GAT TAC ACT AAA ACC CCT GTT AAA ACA GAG TTT      837
Ala Leu Thr Pro Gly Asp Tyr Thr Lys Thr Pro Val Lys Thr Glu Phe
      210      215      220
GGT TAT CAT ATT ATC TAT TTG ATT TCT AAA GAT AGC CCT GTA ACT TAT      885
Gly Tyr His Ile Ile Tyr Leu Ile Ser Lys Asp Ser Pro Val Thr Tyr
      225      230      235      240
ACT TAT GAA CAG GCT AAA CCT ACC ATT AAG GGG ATG TTA CAA GAA AAG      933
Thr Tyr Glu Gln Ala Lys Pro Thr Ile Lys Gly Met Leu Gln Glu Lys
      245      250      255
CTT TTC CAA GAA CGC ATG AAT CAA CGC ATT GAG GAA CTA AGA AAG CAC      981
Leu Phe Gln Glu Arg Met Asn Gln Arg Ile Glu Glu Leu Arg Lys His
      260      265      270
GCT AAA ATT GTT ATC AAC AAG TAATTGATGA GGTGTTATCA TGTTAGTTAA AGGC 1036
Ala Lys Ile Val Ile Asn Lys
      275
AATGAAATTT TATTGAAAGC CCATAAAGAA GGTTATGGGG TGGGGGCGTT TAATTTCTGTG 1096
AATTTTGAAA TGCTAAACGC TATTTTGTGAA GCAGGAAATG AGGAAAATTC CCC      1149

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(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 299 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Signal Sequence
- (B) LOCATION: 1...20
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

```

Met Lys Lys Asn Ile Leu Asn Leu Ala Leu Val Gly Ala Leu Ser Thr
-20      -15      -10      -5
Ser Phe Leu Met Ala Lys Pro Ala His Asn Ala Asn Asn Ala Thr His
      1      5      10
Asn Thr Lys Lys Thr Thr Asp Ser Ser Ala Gly Val Leu Ala Thr Val
      15      20      25
Asp Gly Arg Pro Ile Thr Lys Ser Asp Phe Asp Met Ile Lys Gln Arg
      30      35      40
Asn Pro Asn Phe Asp Phe Asp Lys Leu Lys Glu Lys Glu Lys Glu Ala
      45      50      55      60
Leu Ile Asp Gln Ala Ile Arg Thr Ala Leu Val Glu Asn Glu Ala Lys
      65      70      75
Thr Glu Lys Leu Asp Ser Thr Pro Glu Phe Lys Ala Met Met Glu Ala

```

80						85						90					
Val	Lys	Lys	Gln	Ala	Leu	Val	Glu	Phe	Trp	Ala	Lys	Lys	Gln	Ala	Glu		
95						100						105					
Glu	Val	Lys	Lys	Val	Gln	Ile	Pro	Glu	Lys	Glu	Met	Gln	Asp	Phe	Tyr		
110						115						120					
Asn	Ala	Asn	Lys	Asp	Gln	Leu	Phe	Val	Lys	Gln	Glu	Ala	His	Ala	Arg		
125						130						135					
His	Ile	Leu	Val	Lys	Thr	Glu	Asp	Glu	Ala	Lys	Arg	Ile	Ile	Ser	Glu		
145						150						155					
Ile	Asp	Lys	Gln	Pro	Lys	Ala	Lys	Lys	Glu	Ala	Lys	Phe	Ile	Glu	Leu		
160						165						170					
Ala	Asn	Arg	Asp	Thr	Ile	Asp	Pro	Asn	Ser	Lys	Asn	Ala	Gln	Asn	Gly		
175						180						185					
Gly	Asp	Leu	Gly	Lys	Phe	Gln	Lys	Asn	Gln	Met	Ala	Pro	Asp	Phe	Ser		
190						195						200					
Lys	Ala	Ala	Phe	Ala	Leu	Thr	Pro	Gly	Asp	Tyr	Thr	Lys	Thr	Pro	Val		
205						210						215					
Lys	Thr	Glu	Phe	Gly	Tyr	His	Ile	Ile	Tyr	Leu	Ile	Ser	Lys	Asp	Ser		
225						230						235					
Pro	Val	Thr	Tyr	Thr	Tyr	Glu	Gln	Ala	Lys	Pro	Thr	Ile	Lys	Gly	Met		
240						245						250					
Leu	Gln	Glu	Lys	Leu	Phe	Gln	Glu	Arg	Met	Asn	Gln	Arg	Ile	Glu	Glu		
255						260						265					
Leu	Arg	Lys	His	Ala	Lys	Ile	Val	Ile	Asn	Lys							
270						275											

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1448 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
(B) LOCATION: 118...1314
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CTCTTGAATG	GCGATAAGAC	AAAAATGTCT	TAAATTTTGT	GGTAGCATTT	AGGAATACTT	60
AGGATTTTGT	TTAGTATAAT	TCTAAAATCC	ATTTCAAAAA	ATTAAGGAGA	AATACAA ATG	120
					Met	
					1	
GCA AAA GAA AAG TTT AAC AGA ACT AAG CCG CAT GTT AAT ATT GGA ACC	168					
Ala Lys Glu Lys Phe Asn Arg Thr Lys Pro His Val Asn Ile Gly Thr						
	5			10	15	
ATT GGG CAT GTA GAC CAT GGT AAA ACG ACT TTG AGT GCA GCG ATT TCA	216					
Ile Gly His Val Asp His Gly Lys Thr Thr Leu Ser Ala Ala Ile Ser						
	20			25	30	
GCG GTG CTT TCT TTG AAA GGT CTT GCA GAA ATG AAA GAC TAT GAT AAT	264					
Ala Val Leu Ser Leu Lys Gly Leu Ala Glu Met Lys Asp Tyr Asp Asn						

35	40	45		
ATT GAT AAC GCC CCT GAA GAA AAA GAA AGA GGG ATC ACT ATC GCT ACT			312	
Ile Asp Asn Ala Pro Glu Glu Lys Glu Arg Gly Ile Thr Ile Ala Thr				
50	55	60	65	
TCT CAC ATT GAA TAT GAG ACT GAA AAC AGA CAC TAT GCG CAT GTG GAT			360	
Ser His Ile Glu Tyr Glu Thr Glu Asn Arg His Tyr Ala His Val Asp				
70	75	80		
TGC CCA GGA CAC GCT GAC TAT GTA AAA AAC ATG ATC ACC GGT GCG GCG			408	
Cys Pro Gly His Ala Asp Tyr Val Lys Asn Met Ile Thr Gly Ala Ala				
85	90	95		
CAA ATG GAC GGA GCG ATT TTG GTT GTT TCT GCA GCT GAT GGC CCT ATG			456	
Gln Met Asp Gly Ala Ile Leu Val Val Ser Ala Ala Asp Gly Pro Met				
100	105	110		
CCT CAA ACT AGG GAG CAT ATC TTA TTG TCT CGT CAA GTA GGC GTG CCT			504	
Pro Gln Thr Arg Glu His Ile Leu Leu Ser Arg Gln Val Gly Val Pro				
115	120	125		
CAC ATC GTT GTT TTC TTA AAC AAA CAA GAC ATG GTA GAT GAC CAA GAA			552	
His Ile Val Val Phe Leu Asn Lys Gln Asp Met Val Asp Asp Gln Glu				
130	135	140	145	
TTG TTA GAA CTT GTA GAA ATG GAA GTG CGC GAA TTG TTG AGC GCG TAT			600	
Leu Leu Glu Leu Val Glu Met Glu Val Arg Glu Leu Leu Ser Ala Tyr				
150	155	160		
GAA TTT CCT GGC GAT GAC ACT CCT ATC GTA GCG GGT TCA GCT TTA AGA			648	
Glu Phe Pro Gly Asp Asp Thr Pro Ile Val Ala Gly Ser Ala Leu Arg				
165	170	175		
GCT TTA GAA GAA GCA AAG GCT GGT AAT GTG GGT GAA TGG GGT GAA AAA			696	
Ala Leu Glu Glu Ala Lys Ala Gly Asn Val Gly Glu Trp Gly Glu Lys				
180	185	190		
GTG CTT AAA CTT ATG GCT GAA GTG GAT GCC TAT ATC CCT ACT CCA GAA			744	
Val Leu Lys Leu Met Ala Glu Val Asp Ala Tyr Ile Pro Thr Pro Glu				
195	200	205		
AGA GAC ACT GAA AAA ACT TTC TTG ATG CCG GTT GAA GAT GTG TTC TCT			792	
Arg Asp Thr Glu Lys Thr Phe Leu Met Pro Val Glu Asp Val Phe Ser				
210	215	220	225	
ATT GCG GGT AGA GGG ACT GTG GTT ACA GGT AGG ATT GAA AGA GGC GTG			840	
Ile Ala Gly Arg Gly Thr Val Val Thr Gly Arg Ile Glu Arg Gly Val				
230	235	240		
GTG AAA GTA GGC GAT GAA GTG GAA ATC GTT GGT ATC AGA CCT ACA CAA			888	
Val Lys Val Gly Asp Glu Val Glu Ile Val Gly Ile Arg Pro Thr Gln				
245	250	255		
AAA ACG ACT GTA ACC GGT GTA GAA ATG TTT AGG AAA GAG TTG GAA AAA			936	
Lys Thr Thr Val Thr Gly Val Glu Met Phe Arg Lys Glu Leu Glu Lys				
260	265	270		
GGT GAA GCC GGC GAT AAT GTG GGC GTG CTT TTG AGA GGA ACT AAA AAA			984	
Gly Glu Ala Gly Asp Asn Val Gly Val Leu Leu Arg Gly Thr Lys Lys				
275	280	285		
GAA GAA GTG GAA CGC GGT ATG GTT CTA TGC AAA CCA GGT TCT ATC ACT			1032	
Glu Glu Val Glu Arg Gly Met Val Leu Cys Lys Pro Gly Ser Ile Thr				
290	295	300	305	
CCG CAC AAG AAA TTT GAG GGA GAA ATT TAT GTC CTT TCT AAA GAA GAA			1080	
Pro His Lys Lys Phe Glu Gly Glu Ile Tyr Val Leu Ser Lys Glu Glu				
310	315	320		
GGC GGC AGA CAC ACT CCA TTC TTC ACC AAT TAC CGC CCG CAA TTC TAT			1128	
Gly Gly Arg His Thr Pro Phe Phe Thr Asn Tyr Arg Pro Gln Phe Tyr				
325	330	335		
GTG CGC ACA ACT GAT GTG ACT GGC TCT ATC ACC CTT CCT GAA GGC GTA			1176	
Val Arg Thr Thr Asp Val Thr Gly Ser Ile Thr Leu Pro Glu Gly Val				

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      340              345              350
GAA ATG GTT ATG CCT GGC GAT AAT GTG AAA ATC ACT GTA GAG TTG ATT      1224
Glu Met Val Met Pro Gly Asp Asn Val Lys Ile Thr Val Glu Leu Ile
      355              360              365
AGC CCT GTT GCG TTA GAG TTG GGA ACT AAA TTT GCG ATT CGT GAA GGC      1272
Ser Pro Val Ala Leu Glu Leu Gly Thr Lys Phe Ala Ile Arg Glu Gly
      370              375              380              385
GGT AGG ACC GTT GGT GCT GGT GTT GTG AGC AAT ATT ATT GAA TAATATTAG      1323
Gly Arg Thr Val Gly Ala Gly Val Val Ser Asn Ile Ile Glu
      390              395
CAAAAAGAGA GTTACCATAA AGGGTCATTA TGAAAGTTAA AATAGGGTTG AAGTGTTCTG      1383
ATTGTGAAGA TATCAATTAC AGCACAAACA AGAACGCTAA AACTAACACT GAAAAACTGG      1443
AGCTT                                                                1448

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(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

```

Met Ala Lys Glu Lys Phe Asn Arg Thr Lys Pro His Val Asn Ile Gly
 1              5              10              15
Thr Ile Gly His Val Asp His Gly Lys Thr Thr Leu Ser Ala Ala Ile
      20              25              30
Ser Ala Val Leu Ser Leu Lys Gly Leu Ala Glu Met Lys Asp Tyr Asp
      35              40              45
Asn Ile Asp Asn Ala Pro Glu Glu Lys Glu Arg Gly Ile Thr Ile Ala
      50              55              60
Thr Ser His Ile Glu Tyr Glu Thr Glu Asn Arg His Tyr Ala His Val
      65              70              75              80
Asp Cys Pro Gly His Ala Asp Tyr Val Lys Asn Met Ile Thr Gly Ala
      85              90              95
Ala Gln Met Asp Gly Ala Ile Leu Val Val Ser Ala Ala Asp Gly Pro
      100              105              110
Met Pro Gln Thr Arg Glu His Ile Leu Leu Ser Arg Gln Val Gly Val
      115              120              125
Pro His Ile Val Val Phe Leu Asn Lys Gln Asp Met Val Asp Asp Gln
      130              135              140
Glu Leu Leu Glu Leu Val Glu Met Glu Val Arg Glu Leu Leu Ser Ala
      145              150              155              160
Tyr Glu Phe Pro Gly Asp Asp Thr Pro Ile Val Ala Gly Ser Ala Leu
      165              170              175
Arg Ala Leu Glu Glu Ala Lys Ala Gly Asn Val Gly Glu Trp Gly Glu
      180              185              190
Lys Val Leu Lys Leu Met Ala Glu Val Asp Ala Tyr Ile Pro Thr Pro
      195              200              205
Glu Arg Asp Thr Glu Lys Thr Phe Leu Met Pro Val Glu Asp Val Phe
      210              215              220
Ser Ile Ala Gly Arg Gly Thr Val Val Thr Gly Arg Ile Glu Arg Gly
      225              230              235              240

```

Val	Val	Lys	Val	Gly	Asp	Glu	Val	Glu	Ile	Val	Gly	Ile	Arg	Pro	Thr
				245					250					255	
Gln	Lys	Thr	Thr	Val	Thr	Gly	Val	Glu	Met	Phe	Arg	Lys	Glu	Leu	Glu
				260					265					270	
Lys	Gly	Glu	Ala	Gly	Asp	Asn	Val	Gly	Val	Leu	Leu	Arg	Gly	Thr	Lys
				275					280					285	
Lys	Glu	Glu	Val	Glu	Arg	Gly	Met	Val	Leu	Cys	Lys	Pro	Gly	Ser	Ile
				290					295					300	
Thr	Pro	His	Lys	Lys	Phe	Glu	Gly	Glu	Ile	Tyr	Val	Leu	Ser	Lys	Glu
305					310						315				320
Glu	Gly	Gly	Arg	His	Thr	Pro	Phe	Phe	Thr	Asn	Tyr	Arg	Pro	Gln	Phe
				325							330				335
Tyr	Val	Arg	Thr	Thr	Asp	Val	Thr	Gly	Ser	Ile	Thr	Leu	Pro	Glu	Gly
				340					345					350	
Val	Glu	Met	Val	Met	Pro	Gly	Asp	Asn	Val	Lys	Ile	Thr	Val	Glu	Leu
				355					360					365	
Ile	Ser	Pro	Val	Ala	Leu	Glu	Leu	Gly	Thr	Lys	Phe	Ala	Ile	Arg	Glu
				370					375					380	
Gly	Gly	Arg	Thr	Val	Gly	Ala	Gly	Val	Val	Ser	Asn	Ile	Ile	Glu	
385					390						395				

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CGCGGATCCG AATGAAAAAA AATATCTTAA AT

32

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEO ID NO:70:

CCGCTCGAGT TACTTGTTGA TAACAATTTT

30

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

CGCGGATCCG AATGGCAAAA GAAAAGTTTA AC

32

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CCGCTCGAGT TATTCAATAA TATTGCTCAC

30

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Met	Lys	Glu	Lys	Phe	Asn	Arg	Thr	Lys	Pro	His	Val	Asn	Ile	Gly	Thr
1				5				10						15	
Ile	Gly	His	Val	Asp	His										
				20											

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Ala	His	Asn	Ala	Asn	Asn	Ala	Thr	His	Asn	Thr	Lys	Lys
1				5							10	

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Lys Pro Ala His Asn Ala
1 5

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Ile Asp Lys Gln Pro Lys Ala Lys Lys
1 5

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Phe Trp Ala Lys Lys Gln Ala Glu
1 5

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GTGGAGAACA CACAATGAAA AAAAATATC

29

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GCTAATATTA TTCAATAATA TTGCTCACAA C

31

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GGAGAAATAC AAATGGCAAA AGAAAAG

27

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GCTAATATTA TTCAATAATA TTGCTCACAA C

31

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CATAACGCAA ATAACGCTAC GCAT

24

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GGGAATTCAA AAAAACGAAA AAAACG

26

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

CCCCTCGAGT TAATAGGCAA ACAC

24

What is claimed is:

1. An isolated polynucleotide that encodes:

(i) a polypeptide comprising an amino acid sequence that is homologous to the amino acid sequence of a *Helicobacter* membrane-associated

5 polypeptide, wherein said amino acid sequence of said *Helicobacter* membrane-associated polypeptide is selected from the group consisting of the amino acid sequences as shown:

-in SEQ ID NO:2, beginning with an amino acid in any one of positions -19 to 5, preferably in position -19 or position 1, and ending with an amino acid
10 in position 689 (GHPO 386);

-in SEQ ID NO:4, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 713 (GHPO 789);

-in SEQ ID NO:6, beginning with an amino acid in any one of positions
15 -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 725 (GHPO 1516);

-in SEQ ID NO:8, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 691 (GHPO 1197);

20 -in SEQ ID NO:10, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 652 (GHPO 1180);

-in SEQ ID NO:12, beginning with an amino acid in any one of positions -18 to 5, preferably in position -18 or position 1, and ending with an amino acid
25 in position 673 (GHPO 896);

-in SEQ ID NO:14, beginning with an amino acid in any one of positions

-21 to 5, preferably in position -21 or position 1, and ending with an amino acid in position 619 (GHPO 711);

-in SEQ ID NO:16, beginning with an amino acid in any one of positions -17 to 5, preferably in position -17 or position 1, and ending with an amino acid in position 635 (GHPO 190);

-in SEQ ID NO:18, beginning with an amino acid in any one of positions -19 to 5, preferably in position -19 or position 1, and ending with an amino acid in position 626 (GHPO 185);

-in SEQ ID NO:20, beginning with an amino acid in any one of positions -16 to 5, preferably in position -16 or position 1, and ending with an amino acid in position 467 (GHPO 1417);

-in SEQ ID NO:22, beginning with an amino acid in any one of positions -18 to 5, preferably in position -18 or position 1, and ending with an amino acid in position 673 (GHPO 1414);

- in SEQ ID NO:66, beginning with an amino acid in any one of the positions from -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 279 (GHPO 1360); and

- in SEQ ID NO:68, beginning with an amino acid in position 1 and ending with an amino acid in position 399 (GHPO 750); or

(ii) a derivative of the polypeptide.

2. An isolated polynucleotide that encodes:

(i) a polypeptide comprising an amino acid sequence that is homologous to an amino acid sequence selected from the group consisting of the amino acid sequences as shown:

-in SEQ ID NO:2, beginning with amino acid in position -19 and ending with an amino acid in position 689 (GHPO 386);

- in SEQ ID NO:4, beginning with an amino acid in position -20 and ending with an amino acid in position 713 (GHPO 789);
- in SEQ ID NO:6, beginning with an amino acid in position -20 and ending with an amino acid in position 725 (GHPO 1516);
- 5 -in SEQ ID NO:8, beginning with an amino acid in position -20 and ending with an amino acid in position 691 (GHPO 1197);
- in SEQ ID NO:10, beginning with an amino acid in position -20 and ending with an amino acid in position 652 (GHPO 1180);
- in SEQ ID NO:12, beginning with an amino acid in position -18 and
10 ending with an amino acid in position 673 (GHPO 896);
- in SEQ ID NO:14, beginning with an amino acid in position -21 and ending with an amino acid in position 619 (GHPO 711);
- in SEQ ID NO:16, beginning with an amino acid in position -17 and ending with an amino acid in position 635 (GHPO 190);
- 15 -in SEQ ID NO:18, beginning with an amino acid in position -19 and ending with an amino acid in position 626 (GHPO 185);
- in SEQ ID NO:20, beginning with an amino acid in position -16 and ending with an amino acid in position 467 (GHPO 1417);
- in SEQ ID NO:22, beginning with an amino acid in position -18 and
20 ending with an amino acid in position 673 (GHPO 1414);

- in SEQ ID NO:66, beginning with an amino acid in position -20 and ending with an amino acid in position 279 (GHPO 1360); and
- in SEQ ID NO:68, beginning with an amino acid in position 1 and ending with an amino acid in position 399 (GHPO 750); or
- 25 (ii) a derivative of the polypeptide.

3. The isolated polynucleotide of claim 1, which encodes the mature form of:

(i) a polypeptide comprising an amino acid sequence that is homologous to an amino acid sequence selected from the group consisting of the amino acid sequences as shown:

-in SEQ ID NO:2, beginning with an amino acid in any one of positions -19 to 5, preferably in position -19 or position 1, and ending with an amino acid in position 689 (GHPO 386);

-in SEQ ID NO:4, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 713 (GHPO 789);

-in SEQ ID NO:6, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 725 (GHPO 1516);

-in SEQ ID NO:8, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 691 (GHPO 1197);

-in SEQ ID NO:10, beginning with an amino acid in any one of positions -20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 652 (GHPO 1180);

-in SEQ ID NO:12, beginning with an amino acid in any one of positions -18 to 5, preferably in position -18 or position 1, and ending with an amino acid in position 673 (GHPO 896);

-in SEQ ID NO:14, beginning with an amino acid in any one of positions -21 to 5, preferably in position -21 or position 1, and ending with an amino acid in position 619 (GHPO 711);

-in SEQ ID NO:16, beginning with an amino acid in any one of positions

-17 to 5, preferably in position -17 or position 1, and ending with an amino acid in position 635 (GHPO 190);

-in SEQ ID NO:18, beginning with an amino acid in any one of positions -19 to 5, preferably in position -19 or position 1, and ending with an amino acid in position 626 (GHPO 185);

-in SEQ ID NO:20, beginning with an amino acid in any one of positions -16 to 5, preferably in position -16 or position 1, and ending with an amino acid in position 467 (GHPO 1417);

-in SEQ ID NO:22, beginning with an amino acid in any one of positions -18 to 5, preferably in position -18 or position 1, and ending with an amino acid in position 673 (GHPO 1414);

- in SEQ ID NO:66, beginning with an amino acid in any one of positions

-20 to 5, preferably in position -20 or position 1, and ending with an amino acid in position 279 (GHPO 1360); and

- in SEQ ID NO:68, beginning with an amino acid in position 1 and ending with an amino acid in position 399 (GHPO 750); or

(ii) a derivative of the polypeptide.

4. The isolated polynucleotide of claim 1, 2, or 3, wherein the polynucleotide is a DNA molecule.

5. The isolated polynucleotide of claim 1, which is a DNA molecule that can be amplified and/or cloned by polymerase chain reaction from an *Helicobacter* genome, using either:

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:23, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:25 (unprocessed GHPO 386);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:26, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:28 (unprocessed GHPO 789);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:29, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:31 (unprocessed GHPO 1516);
- 10 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:32, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:34 (unprocessed GHPO 1197);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:35, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:37 (unprocessed GHPO 1180);
- 15 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:38, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:40 (unprocessed GHPO 896);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:41, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:43 (unprocessed GHPO 711);
- 20 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:44, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:46 (unprocessed GHPO 190);
- 25 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:47, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:49 (unprocessed GHPO 185);

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:50, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:52 (unprocessed GHPO 1417);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:53, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:55 (unprocessed GHPO 1414);
- a 5' oligonucleotide primer comprising a sequence as shown in SEQ ID NO:78 and a 3' oligonucleotide primer comprising a sequence as shown in SEQ ID NO:79 (unprocessed GHPO 1360);
- 10 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:24, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:25 (mature GHPO 386);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:27, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:28 (mature GHPO 789);
- 15 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:30, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:31 (mature GHPO 1516);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:33, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:34 (mature GHPO 1197);
- 20 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:36, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:37 (mature GHPO 1180);
- 25 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:39, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:40 (mature GHPO 896);

- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:42, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:43 (mature GHPO 711);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:45, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:46 (mature GHPO 190);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:48, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:49 (mature GHPO 185);
- 10 - a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:51, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:52 (mature GHPO 1417);
- a 5' oligonucleotide primer having a sequence as shown in SEQ ID NO:54, and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:55 (mature GHPO 1414);
- 15 - a 5' oligonucleotide primer comprising a sequence as shown in SEQ ID NO:80 and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:81 (GHPO 750); or
- a 5' oligonucleotide primer comprising a sequence as shown in SEQ ID NO:82 and a 3' oligonucleotide primer having a sequence as shown in SEQ ID NO:79 (mature GHPO 1360).
- 20

6. The isolated DNA molecule of claim 5, which can be amplified and/or cloned by the polymerase chain reaction from a *Helicobacter pylori* genome.

7. The isolated polynucleotide of claim 1, which is a DNA molecule that encodes the mature form or a derivative of a polypeptide encoded by the DNA molecule of claim 5.

8. The isolated polynucleotide of claim 1, which is a DNA molecule
5 that encodes the mature form or a derivative of a polypeptide encoded by the DNA molecule of claim 6.

9. A compound, in a substantially purified form, that is the mature form or a derivative of a polypeptide comprising an amino acid sequence that is homologous to an amino acid sequence of a polypeptide associated with the
10 *Helicobacter* membrane, which is selected from the group consisting of the amino acid sequences as shown:

-in SEQ ID NO:2, beginning with amino acid in position -19 and ending with an amino acid in position 689 (GHPO 386);

-in SEQ ID NO:4, beginning with an amino acid in position -20 and
15 ending with an amino acid in position 713 (GHPO 789);

-in SEQ ID NO:6, beginning with an amino acid in position -20 and ending with an amino acid in position 725 (GHPO 1516);

-in SEQ ID NO:8, beginning with an amino acid in position -20 and ending with an amino acid in position 691 (GHPO 1197);

-in SEQ ID NO:10, beginning with an amino acid in position -20 and
20 ending with an amino acid in position 652 (GHPO 1180);

-in SEQ ID NO:12, beginning with an amino acid in position -18 and ending with an amino acid in position 673 (GHPO 896);

-in SEQ ID NO:14, beginning with an amino acid in position -21 and
25 ending with an amino acid in position 619 (GHPO 711);

-in SEQ ID NO:16, beginning with an amino acid in position -17 and ending with an amino acid in position 635 (GHPO 190);

-in SEQ ID NO:18, beginning with an amino acid in position -19 and ending with an amino acid in position 626 (GHPO 185);

5 -in SEQ ID NO:20, beginning with an amino acid in position -16 and ending with an amino acid in position 467 (GHPO 1417);

-in SEQ ID NO:22, beginning with an amino acid in position -18 and ending with an amino acid in position 673 (GHPO 1414);

10 - in SEQ ID NO:66, beginning with an amino acid in position -20 and ending with an amino acid in position 279 (GHPO 1360); and

- in SEQ ID NO:68, beginning with an amino acid in position 1 and ending with an amino acid in position 399 (GHPO 750); or

(ii) a derivative of said polypeptide.

15 10. The compound of claim 9, which is the mature form or a derivative of a polypeptide encoded by a DNA molecule of claim 5.

11. The compound of claim 9, which is the mature form or a derivative of a polypeptide encoded by a DNA molecule of claim 6.

20 12. A pharmaceutical composition for preventing or treating *Helicobacter* infection in a mammal, said composition comprising a prophylactically or therapeutically effective amount of a compound of claim 9, 10, or 11 and a pharmaceutically acceptable diluent or carrier.

13. The composition of claim 12, further comprising an antibiotic, an antisecretory agent, a bismuth salt, or a combination thereof.

14. The composition of claim 13, wherein said antibiotic is selected from the group consisting of amoxicillin, clarithromycin, tetracycline, metronidazole, and erythromycin.

15. The composition of claim 13, wherein said bismuth salt is selected
5 from the group consisting of bismuth subcitrate and bismuth subsalicylate.

16. The composition of claim 13, wherein said antisecretory agent is a proton pump inhibitor.

17. The composition of claim 16, wherein said proton pump inhibitor is selected from the group consisting of omeprazole, lansoprazole, and
10 pantoprazole.

18. The composition of claim 13, wherein said antisecretory agent is an H₂-receptor antagonist.

19. The composition of claim 18, wherein said H₂-receptor antagonist is selected from the group consisting of ranitidine, cimetidine, famotidine,
15 nizatidine, and roxatidine.

20. The composition of claim 13, wherein said antisecretory agent is a prostaglandin analog.

21. The composition of claim 20, wherein said prostaglandin analog is misoprostil or enprostil.

22. The composition of claim 12, which further comprises a prophylactically or therapeutically effective amount of a second *Helicobacter* polypeptide or a derivative thereof.

23. The composition of claim 22, wherein the second *Helicobacter* polypeptide is a *Helicobacter* urease, a subunit, or a derivative thereof.

24. The composition of claim 12, further comprising an adjuvant.

25. A pharmaceutical composition for preventing or treating *Helicobacter* infection in a mammal, said composition comprising a prophylactically or therapeutically effective amount of a polynucleotide of claim 1, 2, or 3 and a pharmaceutically acceptable carrier or diluent.

26. A pharmaceutical composition for preventing or treating *Helicobacter* infection in a mammal, said composition comprising a prophylactically or therapeutically effective amount of a polynucleotide of claim 5, 6, or 7 and a pharmaceutically acceptable carrier or diluent.

27. A pharmaceutical composition for preventing or treating *Helicobacter* infection in a mammal, said composition comprising a prophylactically or therapeutically effective amount of a polynucleotide of claim 8 and a pharmaceutically acceptable carrier or diluent.

28. A composition comprising a viral vector, in the genome of which is inserted a DNA molecule of claim 4, said DNA molecule being placed under

conditions for expression in a mammalian cell and said viral vector being admixed with a physiologically acceptable diluent or carrier.

29. The composition of claim 28, wherein said viral vector is a poxvirus.

5 30. A composition that comprises a bacterial vector comprising a DNA molecule of claim 4, said DNA molecule being placed under conditions for expression and said bacterial vector being admixed with a physiologically acceptable diluent or carrier.

10 31. The composition of claim 30, wherein said vector is selected from the group consisting of *Shigella*, *Salmonella*, *Vibrio cholerae*, *Lactobacillus*, *Bacille bilié de Calmette-Guérin*, and *Streptococcus*.

15 32. The composition of claim 25, wherein said polynucleotide is a DNA molecule that is inserted in a plasmid that is unable to replicate and to substantially integrate in a mammalian genome and is placed under conditions for expression in a mammalian cell.

33. An expression cassette comprising a DNA molecule of claim 4, said DNA molecule being placed under conditions for expression in a procaryotic or eucaryotic cell.

20 34. A process for producing a compound of claim 9, which comprises culturing a procaryotic or eucaryotic cell transformed or transfected with an

expression cassette of claim 33, and recovering said compound from the cell culture.

35. A pharmaceutical composition for preventing or treating *Helicobacter* infection in a mammal, said composition comprising a
- 5 prophylactically or therapeutically effective amount of an antibody that binds to the compound of claim 9, 10, or 11 and a pharmaceutically acceptable carrier or diluent.

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1 MKK..L-L.L...L...L.AEDDGFYTSVGYQIGEEAAQMV.NTKGIQ.LS GHPO 386
 1 MKKHILSLALGSLVSTLSAEDDGFYTSVGYQIGEEAAQMVNTNTKGIQQLS GHPO 789
 1 MKKHILSLALGSLVSTLSAEDDGFYTSVGYQIGEEAAQMVNTNTKGIQQLS GHPO 1516

MKK..L.L.L...L...L.AEDDGFYTSVGYQIGEEAAQMV.NTKGIQ.LS Consensus

50 DNYE.LNNLL..YSTLNTLIKLSADPSAIN..R.NLG.S..NL...K.NS GHPO 386
 51 DNYENLNNLLTRYSTLNTLIKLSADPSAINAVRENLGAS.KNLIGDKANS GHPO 789
 51 DNYENLNNLLTRYSTLNTLIKLSADPSAINAVRENLGAS.KNLIGDKANS GHPO 1516

DNYE.LNNLL..YSTLNTLIKLSADPSAIN..R.NLG.S..NL...K.NS Consensus

100 PAYQAVLLA.NAAVGLW.V..YA.T.CG-.G.....G...FNN.PGQD GHPO 386
 101 PAYQAVLLAINAAVG.WNV.GY.-T.CG.N.NG.ES.....IFNN.PG.. GHPO 789
 101 PAYQAV.LAINAAVGLWN..GYA-..CG-NGNG.ES..G..IFN..PGQD GHPO 1516

PAYQAV.LA.NAAVG.W....Y....CG.....FN..PG.. Consensus

149 .T.ITCN-....PG.GGP.S..N..K.N.AYQIIQ.AL...-----G.N GHPO 386
 150 ST.ITC.....PG..GPMSI.NFKKLNEAYQILQ.ALKN--G.P.L..N GHPO 789
 149 ST.ITCN-.....G.G..MSI..FKKLNEAYQI.Q.ALKN..G.P.LG.N GHPO 1516

.T.ITC.....G.....S.....K.N.AYQI.Q.AL.....N Consensus

192 G..V.V..N.T.....ING.-----K..G.K..T.....S....I. GHPO 386
 198 ..KVS.V.Y.YTC-----G.....C.....G.K..T.....S.TT.I. GHPO 789
 198 G.KVS.V.YNY.C.....ING.....C..K.....TT... GHPO 1516

...V.V.....G..... Consensus

235 TQ...TI.T.-----.....NNAQ.LL.QAS.II.TLNEACP.F.. GHPO 386
 242I.....T.K.D....AQ.LL.QAS..I.T.NEACP.F.. GHPO 789
 248 TQ...TI.T.....T.K.D-..NNA..LL.QA..I...LN..CP.... GHPO 1516

.....I.....A..LL.QA.....N..CP.... Consensus

279 -----GG...W.G.S..G..CG.F..EISAIQ.MI.NAQE.VAQSKIV GHPO 386
 292 TN.....T.G..CG.F..EISAIQ.MI..AQE.V.Q.... GHPO 789
 297 TN.....GG...W-G.ST.G..C..F..E.S....MI.NAQE..AQSKIV GHPO 1516

.....G..C..F..E.S....MI..AQE...Q.... Consensus

322 SENAQNQN-NLDTGKPFNPYTDASFAQSMLKNAQAQAE.LN.AEQV.KN. GHPO 386
 338 ..N.Q.....GKPFNP.TDASFAQ.ML.NA.AQA.MLNLA.QV.... GHPO 789
 346 SENAQNQN-NLDTGKPFNPYTDASFAQSMLKNAQAQAE.NL.EQV.KN. GHPO 1516

..N.Q.....GKPFNP.TDASFAQ.ML.NA.AQA...N...QV.... Consensus

Alignment of three predicted polypeptides from *H. pylori* that share exact identity at their N-terminus (underlined) with the N-terminal amino acid sequence of the mature native 76 kDa protective antigen. A consensus sequence is indicated in bold. The amino acid sequence of GHPO 386 shares 62% identity in a 733 aa overlap with GHPO 789 and 70% identity in a 745 aa overlap with GHPO 1516. Amino acid positions are numbered to the left of the alignment.

Fig. 1 (page 1 of 2)
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371 E-----...T.FVS..L..C....G---G...G..PG GHPO 386
 388 ---N..N..E..-----NFV..FLA.C..K....G...G..PG GHPO 789
 395 E...N..N..E.....TNFVS.FLA.C--K.G---G.....-- GHPO 1516

.....FV...L..C.....G..... Consensus

400 .VTSNTWGAGCAYV.QTITNL.NSIAHFGTQEQIQQAENIADTLVNFKS GHPO 386
 425 .VT..T...GCAYV.QT.TNL.NSIAHFGTQEQIQQAENIADTLVNFKS GHPO 789
 438 -VTSNTWGAGCAYV..TI..L.NSIAHFGTQEQIQQAENIADTLVNFKS GHPO 1516

.VT..T...GCAYV..T...L.NSIAHFGTQEQIQQAENIADTLVNFKS Consensus

450 RYSELGNTYNSITTALSKVPNAQSLQNVVSKKNNPYSPQGIETNYYLNQN GHPO 386
 475 RYSELGNTYNSITTALSKVPNAQSLQNVVSKKNNPYSPQGIETNYYLNQN GHPO 789
 487 RYSELGNTYNSITTALSKVPNAQSLQNVVSKKNNPYSPQGIETNYYLNQN GHPO 1516

RYSELGNTYNSITTALSKVPNAQSLQNVVSKKNNPYSPQGIETNYYLNQN Consensus

500 SYNQIQTIQELGRNPFRKVGIVNSQTNGAMNGIGIQVGYKQFFGQKRK GHPO 386
 525 SYNQIQTIQELGRNPFRKVGIVNSQTNGAMNGIGIQVGYKQFFGQKRK GHPO 789
 537 SYNQIQTIQELGRNPFRKVGIVNSQTNGAMNGIGIQVGYKQFFGQKRK GHPO 1516

SYNQIQTIQELGRNPFRKVGIVNSQTNGAMNGIGIQVGYKQFFGQKRK Consensus

550 WGARYYGFFDYNHAFIKSSFFNSASDVWTYGFGADALYNFINDKATNFLG GHPO 386
 575 WGARYYGFFDYNHAFIKSSFFNSASDVWTYGFGADALYNFINDKATNFLG GHPO 789
 587 WGARYYGFFDYNHAFIKSSFFNSASDVWTYGFGADALYNFINDKATNFLG GHPO 1516

WGARYYGFFDYNHAFIKSSFFNSASDVWTYGFGADALYNFINDKATNFLG Consensus

600 KNNKLS.GLFGGIALAGTSWLNSEYVNLATVNNVYNKMNVANFQFLFNM GHPO 386
 625 KNNKLSLGLFGGIALAGTSWLNSEYVNLATVNNVYNKMNVANFQFLFNM GHPO 789
 637 KNNKLSLGLFGGIALAGTSWLNSEYVNLATVNNVYNKMNVANFQFLFNM GHPO 1516

KNNKLS.GLFGGIALAGTSWLNSEYVNLATVNNVYNKMNVANFQFLFNM Consensus

650 GVRMNLARSKKKGSDHAAQHGIELGLKIPTINTNYYSFMGAELKYRRLYS GHPO 386
 675 GVRMNLARSKKKGSDHAAQHGIELGLKIPTINTNYYSFMGAELKYRRLYS GHPO 789
 687 GVRMNLARSKKKGSDHAAQHGIELGLKIPTINTNYYSFMGAELKYRRLYS GHPO 1516

GVRMNLARSKKKGSDHAAQHGIELGLKIPTINTNYYSFMGAELKYRRLYS Consensus

700 VYLNXYVFAY GHPO 386
 725 VYLNXYVFAY GHPO 789
 737 VYLNXYVFAY GHPO 1516

VYLNXYVFAY Consensus

Fig. 1 (page 2 of 2)

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MKKLLL-LL-----AEDDGFYTSVGYQIGEEAQMNTKGI--QL		Consensus
1	MKK.LL-L.....AED.GF..S.GYQIGEEAQMV...G...L	GHPO 1180
1	MKK.LL-L--.....AEDDGFYTSVGYQIGEA.Q.V...G...L	GHPO 896
1	MKK.LL-L--.....AEDDGFYTSVGYQIGEA.Q.V...G...L	GHPO 1414
1	MKK..L.L.....-AED.GF..S.GYQIGE.AQMV.....L	GHPO 1197
1	MKK.....L.....AED.G...SVGYQIGEA.Q.V.....L	GHPO 711
1	MKK..L-L.....AED.GF..S.GYQIGE..QMV...G...L	GHPO 185
1	MKK..L-L.....AED.GF..S.GYQIGEA.QMV...G...L	GHPO 190
1	MKK..L-----AE.DG...SVGYQIGEA.Q.V...G....	GHPO 1417
SDNYE-LNNLL---YSTLNTLIKLSADPSAIN-----RNLGSNLKN		Consensus
50	SD.YE.L.NLL....LN.....PS.IN.....L.....N	GHPO 1180
48	.D.Y..L.NLL..Y..LN.L..L...PSAI.....NL.S....	GHPO 896
48	.D.Y..L.NLL..Y..LN.L..L...PSAI.....NL.S....	GHPO 1414
50	SD.YE.L.NLL..YS.LNTL...SADP.AIN.....NL....KN	GHPO 1197
51	SD.YE.L..LL.----N.....S.....IN.....TL....N	GHPO 711
49	...YE.L.....L...I.....A.N.....N...N...	GHPO 185
47	...YE.L...L...L...I.....N.....N.....	GHPO 190
46	S..YE.L.NLL..Y..L.---S...S.....L--....	GHPO 1417
SPAYQAV-LA-NAAVG-W-----Y---CG-----FN--PG-		Consensus
100	SPAYQAV.LA.NAAVG.W....-Y...-CG.....--F...PG.	GHPO 1180
98	SPAYQAV.LA.NAAVG.W....-....-CG.....--F...P..	GHPO 896
98	SPAYQAV.LA.NAAVG.W....-....-CG.....--F...P..	GHPO 1414
100	SPAYQAV.LA.NAA.G.W....-Y...CG.....F...P...	GHPO 1197
96	SPAYQA..LA.....G.W....-Y...-CG.....F.....	GHPO 711
99	SP.Y.....W.....	GHPO 185
97	SP...AV.....G.W.....-.....P...	GHPO 190
92	..A.QAV..A...AV..W....-.....	GHPO 1417
----TITCGS-----KNAYQ---		Consensus
145	...I.C.....-.....AYQ...	GHPO 1180
144	...T.TC.....-.....AYQ...	GHPO 896
144	...T.TC.....-.....AYQ...	GHPO 1414
149	...TI.C.....-.....AYQ...	GHPO 1197
144	...TI.CG.....-.....AYQ...	GHPO 711
146	-----C.....	GHPO 185
146	-----C.....	GHPO 190
118	-----Q...	GHPO 1417

Alignment of eight related polypeptides from *H. pylori* that share significant identity with the consensus sequence determined for the 76 kDa family, a member of which has been determined to be protective in animal models. The amino acid sequence from GHPO 386 shares 53% identity in a 672 aa overlap with GHPO 1180, 51% identity in a 691 aa overlap with GHPO 896, 51% identity in a 691 aa overlap with GHPO 1414, 63% identity in a 711 aa overlap with GHPO 1197, 44% identity in a 640 aa overlap with GHPO 711, 37% identity in a 645 aa overlap with GHPO 185, 36% identity in a 652 aa overlap with GHPO 190 and 41% identity in a 483 aa overlap with GHPO 1417. Amino acid positions are numbered to the left of the alignment. Gaps (-) have been introduced to maximize alignment. Absolute identity shown only (all other residues identified by a dot).

Fig. 2 (page 1 of 4)

SUBSTITUTE SHEET (RULE 26)

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-----IQALNVVG-----		Consensus
181L..G.....	GHPO 1180
179ALN.....	GHPO 896
179ALN.....	GHPO 1414
184AL.....	GHPO 1197
194LN..G.....	GHPO 711
173L.....	GHPO 185
177L.....	GHPO 190
138	GHPO 1417
-----IALLQANCP-----		Consensus
209	-----I..L..NCP.....	GHPO 1180
229L...P.....	GHPO 896
229L...P.....	GHPO 1414
231L...CP.....	GHPO 1197
228L...CP.....	GHPO 711
202K...IA.....	GHPO 185
212K...IS.....	GHPO 190
146	-----I..LQ..CP-----	GHPO 1417
-----GC--F-----ESMIAQEQNQ		Consensus
248	-----C..F.....Q..Q..	GHPO 1180
273	-----A..Q..	GHPO 896
273	-----A..Q..	GHPO 1414
277	-----C..F.....E...Q...	GHPO 1197
268	-----C..F.....Q..	GHPO 711
238	-----Q..	GHPO 185
245	-----Q..	GHPO 190
173	-----C..F.....A.....	GHPO 1417
GKP-----FNPTD-ASFAQ-MLNAAQAN-----		Consensus
295	..P.....D-..FAQ.MLN.AQAQ.....	GHPO 1180
318D-..FAQ.MLN.A.AQ.....	GHPO 896
318D-..FAQ.MLN.A.AQ.....	GHPO 1414
327D-A.FAQ.M...A.AQ.....	GHPO 1197
317P.....AQ.ML..AQ.Q.....	GHPO 711
273FA.....AQ.....	GHPO 185
280FA.....AQ.....	GHPO 190
219	GHPO 1417

Fig. 2 (page 2 of 4)

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	-----QVFVL-----C-----GVTT-----GCAYV-----	Consensus
341F.....C.....GVT.....GCAYV.....	GHPO 1180
365-.....C.....T.....GCA.V.....	GHPO 896
365-.....C.....T.....GCA.V.....	GHPO 1414
374FV.....C.....VT.....GCAYV.....	GHPO 1197
365K.....C.....GCA.V.....	GHPO 711
316K-.....G.T.....	GHPO 185
323K-.....G.T.....	GHPO 190
237V-----	GHPO 1417
	-----QVFVL-----C-----GVTT-----GCAYV-----	Consensus
341F.....C.....GVT.....GCAYV.....	GHPO 1180
365-.....C.....T.....GCA.V.....	GHPO 896
365-.....C.....T.....GCA.V.....	GHPO 1414
374FV.....C.....VT.....GCAYV.....	GHPO 1197
365K.....C.....GCA.V.....	GHPO 711
316K-.....G.T.....	GHPO 185
323K-.....G.T.....	GHPO 190
237V-----	GHPO 1417
	-TLN-SIAHFGTQEQIQQAE NIADTLVNFKSRYS ELGNTYN---SITTA	Consensus
386	..LN.S.AHFGTQ..QI.Q.E..A.T..F.....L.NTYN---SITT.	GHPO 1180
405	..L....A..G---Q..Q....A.T..NFK.....L.....-I...	GHPO 896
405	..L....A..G---Q..Q....A.T..NFK.....L.....-I...	GHPO 1414
424	..L..SIAHFG.Q...I..A.N.A.TL.NF...Y..LG.....-SIT.A	GHPO 1197
412	..L..S.A.F..Q..QI.QA.N.A.TL-----	GHPO 711
357	.T.....G---A...A...N.KS...E...YN.....	GHPO 185
364	.T.....G---A...A...N.KS...E...YN.....	GHPO 190
250	..L-----G.....-I...	GHPO 1417
	LSKVPNAQ-SLQNVVSKKNPYS PQGIETNYL NQNSYNQIQ TINQELGR	Consensus
433	.S..PN..-L.N..S..NP..P.G...Y..NQ..Y.Q....QELG.	GHPO 1180
449	.S..PNA.-SLQN.....NP.SP.G..T-Y.L...YNQ.QTI.QELG.	GHPO 896
449	.S..PNA.-SLQN.....NP.SP.G..T-Y.L...YNQ.QTI.QELG.	GHPO 1414
471	LS..P.AQ-SLQNVVSKK.NP.SPQGI..NYY...N...Q.Q...QELG.	GHPO 1197
440	-----QELG.	GHPO 711
404	.SK.P..Q.....V.....P.....NY..N.....	GHPO 185
411	.SK.P..Q.....V.....P.....NY..N.....	GHPO 190
264	L.K.P....Q..VS...YS-----Y.LN...Y...QT...E.G.	GHPO 1417

Fig. 2 (page 3 of 4)

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NPFRKVGIVN-SQTNNGAMNGIGIQVGYKQFFGQKRKWGARYYGFDDYNH		Consensus
482	NPFR.VG...-SQTNNGAMNGIG.Q.GYKQFFG.KR.WG.RYYGFFDYNH	GHPO 1180
497	NPFR..G...-.Q.NNGAMNGIG.QVGYKQFFG.KR.WG.RYYGFFDYNH	GHPO 896
497	NPFR..G...-.Q.NNGAMNGIG.QVGYKQFFG.KR.WG.RYYGFFDYNH	GHPO 1414
520	NPFR..G....S.TNNGAMNGIG.QVGYKQFFG...WGARYYGF.DYNH	GHPO 1197
445	NPFR..G....S.TNNGA.NG.G.QVGYKQFFG.K..WG.RYYGFFDYNH	GHPO 711
454	NPF.KVG....-Q.NNGA.NG.G.QVGYKQFFG...WG.RYYGFFDYNH	GHPO 185
461	NPF.KVG....-Q.NNGA.NG.G.QVGYKQFFG...WG.RYYGFFDYNH	GHPO 190
304	NPFR.VG..N.-Q.NNGAMNG.G.Q.GYKQFFG...FG.RYY.FFDYNH	GHPO 1417
AFIKSSFFNSASDVWTYGFADALYNFINDKATNFLGKNNKLS-GLFGGI		Consensus
531	A.IKSSFFNSASDV.TYG.G.D.LYNFINDKAT----KNNK.S.G.FGGI	GHPO 1180
546	A.IKS.FFNSASDVWTYG.G.DALYNFINDK.TNFLGKNNKLS.GLFGG.	GHPO 896
546	A.IKS.FFNSASDVWTYG.G.DALYNFINDK.TNFLGKNNKLS.GLFGG.	GHPO 1414
570	..KS.FFNS.SDVWTYG.G.D.L.NFINDKAT----K.NK.S.G.FGGI	GHPO 1197
495	A.IKS.FFNSASDVWTYG.G.D.L.NFINDK.TNFLGKNNK.S.G.FGGI	GHPO 711
503	..IKSSFFNS.SD.WTYG.G.D.L.N.IND..T.---KNNKLS.GLFGGI	GHPO 185
510	..IKSSFFNS.SD.WTYG.G.D.L.N.IND..T.---KNNKLS.GLFGGI	GHPO 190
353	A.IKS.FFNSAS.V.TYG.G.D.L.NFIN....----.KN.K.S.G.FGGI	GHPO 1417
ALAGTSWLNSEYVNLATVNNVYNAMNVANFQFLFNMGVRMNLARSKKKG		Consensus
577	ALAGTSWLNS.YVNLAT.NN.Y.AKMNVANFQFLFN.G.RMNLA..KKK.	GHPO 1180
596	ALAGTSWLNS..VNL...N..YNA.....NFQFLF..G.RMNLAR.KKK.	GHPO 896
596	ALAGTSWLNS..VNL...N..YNA.....NFQFLF..G.RMNLAR.KKK.	GHPO 1414
616	.LAGTSWLNS.YVNL.VNN.Y.AK.N..NFQFLFN.G.R.NLAR.K..G	GHPO 1197
545	ALAGTSWLNS..VNL.T..NVY.AK.N.ANFQFLFN.G.R.NLAR.KKK.	GHPO 711
550	.LAGT.WLNS.YVNL...NN.Y.AK.N..NFQFLFN.G.R.NLA...KK.	GHPO 185
557	.LAGT.WLNS.YVNL...NN.Y.AK.N..NFQFLFN.G.R.NLA...KK.	GHPO 190
399	ALAGT.WLNS...NL.T.N..Y.AK.N..NFQFLFN.G.R.-----	GHPO 1417
SDHAAQHGIELGLKIPTINTNYYSFMGAELKYRRLYSVYLNIVFAY		Consensus
627	SDH.AQHG.ELG.KIPTINTNYYS..G..L.YRRLYSVYLNIVFAY	GHPO 1180
646	SDHAAQHGIELG.KIPTINTNYYSFMGA.L.YRR.YS..LNYVFAY	GHPO 896
646	SDHAAQHGIELG.KIPTINTNYYSFMGA.L.YRR.YS..LNYVFAY	GHPO 1414
666	.DH.AQHG.ELG.KIPTINTNYYS..G..L.YRRLYSVYLNIVFAY	GHPO 1197
595	S.HAAQHGIELG.KIPTINTNYYSF...L.YRRLYSVYLNIVFAY	GHPO 711
600	S.H.AQHGIELG.KIPTI.TNYYSF.G..L.YRRLYSVYLNIVFAY	GHPO 185
607	S.H.AQHGIELG.KIPTI.TNYYSF.G..L.YRRLYSVYLNIVFAY	GHPO 190
440	--....HG.ELG.KIPTINTNYYSFMGA.L.YRRLYSVY.NYV.AY	GHPO 1417

Fig. 2 (page 4 of 4)

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/06421**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : 514/ 25, 44, 53, 147; 435/7.32; 424/150.1, 242.1, 451, 653

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/ 25, 44, 53, 147; 435/7.32; 424/150.1, 242.1, 451, 653

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P ----- Y,P	TOMB, J. et al. The complete genome sequence of the gastric pathogen <i>Helicobacter pylori</i> . Nature, 07 August 1997. Vol. 388, pages 539-547, see entire document.	1-12 ----- 13-35
X,P	WO 97/12908 A1 (PASTEUR MERIEUX SERUMS AND VACCINS) 10 April 1997, see English abstract, and entire document.	9-12
X Y Y	WO 97/11182 A1 (MAX-PLANCK-GESELLSCHAFT ZUR FORDERUNG DER WISSENSCHAFTEN E.V.) 27 March 1997, see English abstract and entire document.	1-12 13-35
Y	US 5,116,821 A (RANDALL et al.) 26 May 1992, see abstract, col. 6, lines 22-68, col. 7, lines 1-41, see entire document.	13-22

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 01 JULY 1998	Date of mailing of the international search report 21 AUG 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GINNY PORTNER Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/06421

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,128,140 A (CHAPURA et al.) 07 July 1992, see abstract, col. 3-5, see entire document.	13-22
Y	US 5,447,923 A (CATRENICH et al.) 05 September 1995, col. 3, 6, and entire document.	13-22
Y,P	US 5,620,964 A (ROTH et al.) 15 April 1997, see abstract, columns 6-8 and entire document.	13-22
Y	US 5,514,660 A (ZOPF et al.) 07 May 1996, column 7-10, see entire document.	13-22
Y,E	US 5,753,630 A (ZOPF et al.) 19 May 1998, column 5, lines 23-31, 7-10, see entire document.	13-22
Y	US 5,476,669 A (BORODY) 19 December 1995, column 1-4, see entire document.	13-22
Y	US 5,610,060 A (WARD et al.) 11 March 1997, column 8, lines 15-41, see entire document.	25-34
A	US 5,567,594 A (CALENOFF) 22 October 1996, see abstract, columns 13-16, and entire document.	1-35
Y	US 5,563,039 A (GOEDEL et al.) 08 October 1996, see column 2, lines 32-46, column 5, lines 8-14 and entire document.	25-34
X	US 5,258,178 A (CORDLE et al.) 02 November 1993, see abstract, column 4, lines 12-18, 50-58 and entire document.	35
X	US 5,538,729 A (CZINN et al.) 23 July 1996, see column 4, lines 24-25, 44-67 and entire document.	9-12, 35
-		----
Y		30-32
Y	WO 96/40893 A1 (ASTRA AKTIEBOLAG) 19 December 1996, see pages 74-118, and entire document.	1-35
X	FAUCHERE, J. et al. Adherence of Helicobacter pylori cells and their surface components to HeLa cell membranes. Microbial Pathogenesis. 1990, Vol. 9, pages 427-439, see pages 430-431 and entire document.	9-12, 22-23
X	DROUET, E.B. et al. Partial characterization of an external polysaccharide of Helicobacter pylori by using an immunoglobulin M monoclonal antibody. Infection and Immunity, June 1993. Vol. 61, No. 6, pages 2732-2736, especially pages 2734-2735.	9-12, 22

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUSSON, M. et al. Iron acquisition by Helicobacter pylori: Importance of Human Lactoferrin. Infection and Immunity, June 1993, Vol. 61, No. 6, pages 2694-2697, especially page 2697.	9-12, 22
X	ILLINGWORTH, D.S. et al, Siderophore production and iron-regulated envelope proteins of Helicobacter pylori. Zentralblatt fur Bakteriologie, September 1993, Vol. 280, No. (1-2), pages 113-119, especially abstract and page 118.	9-12, 22
Y,P	US 5,679,769 A (DANISHEFSKY et al.) 21 October 1997, column 20, lines 45-65, column 2, lines 26-40.	12-21
A	BOREN, T et al. Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. Science. 17 December 1993, Vol. 262, pages 1892-1895, especially page 1893.	1-35
A	SHERBURNE, R. et al. Helicobacter pylori expresses a complex surface carbohydrate, Lewis X. Infection and Immunity. December 1995, pages 4564-4568, especially page 4564-4565.	1-35
A	WADSTROM, T. Microbial adhesion new concepts for development of antiadhesion strategies to combat bacterial infections. ACTA Microbiological Hungary. 22-24 August 1991, Vol. 38, No. (3-4), pages 164-165, especially page 165.	9-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/06421**Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A01N 43/04, 59/16; A61K 9/48, 31/70, 31/715, 39/02, 39/40; G01N 33/554, 33/569

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

GENEBANK, APS, Dialog

search terms: specific SEQ ID NO., bismuth?, pylori, helicobacter, adjuvant?, urease?, prostagland?, enprostil?, misoprostil?, proton (3a) pump, subcitrate?, subsalicylate?, antisecret?, antibiotic?

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-8, 25-34, drawn to no fewer than 84 polynucleotides, vectors containing the polynucleotides, organisms transformed with the polynucleotides, polynucleotides encoding fragments of the polypeptides encoded by the no fewer than 84 different polynucleotides, vaccines and methods of treating a host with the polynucleotides.

Group II, claim(s) 9-24, drawn to polypeptides encoded by the polynucleotides mentioned in Group I.

Group III, claim(s) 35, drawn to a composition comprising antibodies for the treatment of infection.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

Group I contains a separate polynucleotide species for each sequence mentioned. Therefore, there is a minimum of 84 species. Group II contains at least one polypeptide for each polynucleotide sequence mentioned. Therefore, there is a minimum of 19 species in Group II.

For either Group that applicant elects, a total of 10 (ten) specified sequences will be searched and no more than 4 specified sequences will be searched for each additional fee paid; if no additional fee is paid and no election indicated, the first 10 sequences will be searched.

The inventions listed as Groups I, II or III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the polypeptide encoding polynucleotides, vectors containing the polynucleotides, organism transformed with said polynucleotides and methods of use are materially different from each other and are therefore independent from the polypeptide of Group II. Additionally, none of the products or methods of Group I is needed to make the polypeptide of Group II. The antibodies of Group III are structurally and functional different from the polynucleotides and polypeptides of Groups I and II and therefore, evidence different special technical features which have differing functions and effects. The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: There is no relationship between or among the various nucleotide and amino acid sequences mentioned in the claims.

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